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Active Avoidance Conditioning in Certain Anura

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ACTIVE AVOIDANCE CONDITIONING IN CERTAIN ANURA

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VITA

The author, Frank James Cann, is the son of Frank Cann and Mary (Chernetski) Cann. He was born July 6, 1944, in Newark, New Jersey.

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On July 5, 1969, he married Audrey Joyce Vogt and had a son, Frank Garrett Cann, on November 27, 1970.

DEDICATION

To those who love: since you bring great joy.

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INTRODUCTION

The study of amphibian behavior has been neglected when compared to the behavioral research currently in vogue with other vertebrate species. As a consequence of this general lack of behavioral research, there is a paucity of research on the learning capabilities of frogs and toads. Consequently, there is little evidence to prove the existence of learning in anurans. Meanwhile learning has been clearly demonstrated in the teleost fishes (Behrend, 1962) and this has provided a stimulus for a considerable amount of behavioral research with this class.

In attempting to understand the mechanisms of learning, researchers are using even simpler animal systems than the fishes in their investigations. Perhaps the Anura and Caudata orders of the class Amphibia will eventually be examined thoroughly; but at present they remain largely unexplored.

The current concepts of memory do not favor a single fundamental mechanism. Bullock (1966) emphasizes that the many varieties of sensory and motor apparatuses which exist in the animal kingdom mitigate the possibility of a unitary theory for the mechanism of memory formation. However, the possibility that a general electrophysiological property of neurons could be found to be part of a mechanism for memory, still should be considered (Morrell, 1961). It would be quite exciting if such a property would be as ubiquitous in the transfer of behavioral information as DNA is for the transfer of genetic information.

An objective of this introduction is to delineate the

questionability of some of the early work in amphibian learning studies. Another objective is to summarize the previous knowledge concerning the learning abilities of Amphibia and to show how that knowledge relates to the present investigation. In addition, this introduction will cite what the author believes to be the four strongest published arguments for the existence of learning in certain anuran species. Three of these arguments have developed coincidentally and independently of the investigation presented here. I shall defer until the discussion this author's observations and how they compare with the observations of earlier investigators.

Studies of learning in anura have emphasized: maze learning (Yerkes, 1903a; Burnett, 1912; Buytendijk, 1918b; Cummings, 1910); feeding associations (Yerkes, 1903a; Franz, 1927; Burnett, 1912; Abbott, 1884; Schaeffer, 1911; Buytendijk, 1918a; Brower and Brower, 1960; Schmidt, personal communication; Razwilowska, 1927; Munn, 1940; Nobel, 1942; Vandel, 1927; Van Bergeijk, 1967; Boice, 1969; Haecker, 1912), and conditioned avoidance behavior (Yerkes, 1903a; Burnett, 1912; Munn, 1940; Bajandurow and Pegel, 1932; Kleerekoper and Sibabin, 1959; McGill, 1960; Martof, 1962; Ray, 1967 and Boice, 1970). These areas of involvement will be discussed in order. Moreover, early in the history of such learning studies, some unusual basic traits of frogs and toads became apparent. These unusual aspects of frog behavior shall

also be discussed in the subsequent paragraphs.

LEARNING STUDIES WITH MAZES

Some of the earliest studies on learning in Amphibia used simple mazes. Yerkes (1903a) constructed a rectangular shaped maze. One end of Yerkes' maze had a pool of water. This pool was partially separated from the rest of the maze by a glass partition. Thus, depending upon its direction of movement, a frog could either jump into the partition or to the right of the partition and into the water. In front of the glass partition and centrally located in the maze was a triangular shaped barrier. The height of the barrier was the same as the sides of the maze. The triangular barrier had its apex pointed towards the starting box. Thus the barrier created two walls and two alleys. These alleys when viewed with the starting area created a "Y" maze. The left side of the barrier was colored red and was the same color as the maze wall which it faced. Likewise, the right side of the barrier was colored white and it also matched the maze wall which it faced.

The maze therefore presented the test animal with two choices. The first choice was either to the left or to the right. This first choice also had a color cue produced by the maze wall and the triangular barrier. The second choice was either straight ahead into the glass, or a 90° turn to the right which would lead the frog to the pool of water. Yerkes' (1903a) investigation revealed that a frog could learn his way through

such a simple "Y" maze. It took fifty to one hundred trials for a Rana pipiens to learn the maze. Color perception as well as tactile information seemed to be necessary for learning to occur (Yerkes, 1903a). The memory of experience when tested seemed to last at least one month.

However, there may be a few overstatements embodied in Yerkes' conclusion. Yerkes stated that he only used the most active animals. Thus at the outset, his experiment was not representative of the species. Secondly, a learning curve was not plotted and statistics were not used to quantify the degree of error in his experiment. Thirdly, he could not demonstrate extinction and relearning. This is evidenced by the fact that once the animals had learned the appropriate turns they could not find the end of the maze, a tank of water, when the cue pattern was reversed. Yerkes also concluded that the pattern which was formed by colored cards that lined the walls was used by the frog in learning the maze. However, when these colored cards were switched along with those of the barriers the animal no longer made the first choice correctly. Hypothetically, this first choice would have merely been a choice of color, however, the animal followed the same pathway it had previously "learned". This leads one to suspect that the perception of color was not important in the choice of direction and that some other sensory phenomena was responsible for the behavior. One wonders if this learning could have been a case of celestial orientation to an

artificial light source such as has been reported by Landreth and Ferguson (1968).

In another simple maze experiment designed to test the formation of associations Yerkes (1903a) placed frogs in a box from which they had to escape. The frogs had to learn that they could not leap straight ahead to escape sinceit was blocked by a glass plate. This plate of glass was only 2/3 the height of the box and therefore the frog, in order to escape, had to jump over the glass partition. Yerkes (1903a) concluded that no associations were formed during this precedure. Electric shock was added and investigated as a motivating stimulus. With shock the behavioral responses deteriorated because the frogs' violent struggles to escape led to increased escape times without any observable adaptation.(Yerkes, 1903a).

Unfortunately, Yerkes generalized his results and implied that learning was a racial characteristic. Yerkes presented evidence for learning by discussing in depth the experience of one animal in the "Y" maze, and he clearly omitted discussion of any other animals' performance. However, in his conclusions, he speaks about green frogs as a race, but since racial characteristics are readily identifiable traits, they cannot be limited to only a few members of the species. Yerkes realized this and specified his use of very active animals in the text; however, he disregarded the fact in his conclusions when he generalized "Y" maze learning to be a racial characteristic of frogs.

Further research with mazes was carried out in decerebrate frogs. In Burnett's (1912) investigation a modified "Y" maze of Yerkes was used eliminating the first choice and maintaining only the last choice. Therefore, this simple maze required only one choice of direction by the experimental subject. It was subsequently found that decerebrate frogs could not learn the maze. It was concluded that the cerebral hemispheres are necessary for learning to occur (Burnett, 1912).

However Burnett's conclusion was based on research containing serious oversights since he did not describe his surgical procedure nor did he present an anatomical verification of the lesions which he reported to interfere with learning.

Buytendijk (1918b) performed an experiment similar to one of Yerkes (1903a). He utilized a box with the escape route blocked by a glass plate. This glass plate was 2/3 the height of the box and the frog had to learn to jump over the glass to escape. Buytendijk found that the toad Bufo clamitans could learn to jump over the barrier in as few as nine trials. He also found that the toads maintained certain movements which were seemingly useless in solving the escape problem.

Maze learning studies have led to the concept of a muscular memory (Buytendijk, 1918b; Cummings, 1910; Nobel, 1942). Cummings in his studies with British newts found that seemingly unimportant motor movements persisted in animals even after they had achieved some quantifiable level of learned performance.

According to Cummings these movements were unrewarded, unpunished, and seemingly did not aid in producing the appropriate responses. Cummings labeled this phenomenon, muscular memory. Buytendijk observed a similar phenomenon in his escape training of toads and considered it to be persistent unrewarded behavior. Nobel (1942) gave a functional significance to this muscular memory by stating that it helps the toad find his way back to his territory after feeding.

Recent experiments and definitions may alter Cummings' concept of a "muscular memory". The problem of homing behavior in Anura has recently been resolved by the findings of Landreth and Ferguson (1968). Landreth and Ferguson have shown that toads and frogs can move to a particular space using the sun as a navigational guide. Moreover the toads can adjust their movements taking into account the difference in the sun's position throughout the day. Thus Nobel's functional significance of the "muscular memory" is no longer necessary to explain homing behavior. I believe that it would be very difficult to differentiate the behavior associated with "muscular memory" from stereotypic behavioral patterns.

Other studies using maze learning techniques involved the use of tadpoles. Munn (1940) combined a "T" and a "Y" maze technique with a shock avoidance technique to investigate the learning abilities of Hyla cinerea, Hyla versicolor and Rana catesbeiana tadpoles. He found that associations were made with

difficulty and only one tadpole showed any consistent response which resembled learning.

In summary the studies with mazes attempted to demonstrate an ability to learn in normal frogs and toads (Yerkes, 1903a; Buytendijk, 1918b). Other investigators claimed the relative absence of an ability to learn in tadpoles (Munn, 1940) and in decerebrated frogs (Burnett, 1912). Unfortunately, these experiments were not well controlled and some doubt as to the proof of maze learning abilities in frogs and toads could easily remain in the mind of a critical observer.

LEARNING EXPERIMENTS INVOLVING FEEDING ASSOCIATIONS

Vision plays an important part in the feeding behavior and development of feeding associations in Anura. Certain species of frogs and toads require moving food to entice them to eat (Yerkes, 1903a, and Franz, 1927). The feeding response of frogs and toads is composed of at least five movements: head turning, body orientation, prey stalking, prey catching and swallowing. Prey movements are the primary stimuli for releasing the chain of reflexes comprising feeding behavior. Movement is such an important factor that in its absence a frog may starve to death even in the presence of an abundance of non-moving food (Yerkes, 1903a).

In further experiments the role of the cerebrum in feeding responses was investigated. Burnett (1912) observed the feeding behavior of decerebrated and intact frogs. Burnett reported

that the decerebrate animals were more active feeders than normal animals. He claimed that the decerebrated animals showed less reflex inhibition of movement and this enabled them to eat more flies than the intact frogs. Burnett hypothesized that a lack of fear was causally related to the observed behavior of the lesioned animals.

Ewert (1970) has demonstrated that removal of the cerebrum will enhance the disinhibition of prey-catching behavior of pretectally lesioned animals. Since no anatomical evidence was presented by Burnett, one might speculate that Burnett actually produced combined decerebrated and pretectally lesioned animals rather than decerebrated animals.

Ewert hypothesized that the pretectal area functions to inhibit snapping reflexes evoked by visual stimuli being integrated in the tectal regions. Ewert further hypothesized that the pretectal area is then involved in the production of visually evoked avoidance responses in the toad. Therefore, Ewert's mechanism involving a loss of inhibition might be more apropos than Burnett's "lack of fear". Furthermore, since no one has quantitatively measured fear in amphibia Burnett's hypothesis is based upon an unquantified and undefined variable.

The importance of the frog's visual system in feeding behavior and consequently in the formation of a feeding association is also evident in the work of Sperry (1944). Sperry severed the optic nerves and rotated the frogs eyeball 180°. Upon

operative recovery the frogs were tested for their reactions to visual stimuli. It was discovered that the nerves had regenerated to make their original connections in the tectum. However, the frog's visual perception was still relative to the retinal field which was stimulated. In other words the frog's responses were 180° opposite to what they should have been for the particular stimulus. It was also discovered that for at least seventy days after the initial recovery of vision frogs could not learn to readjust their behavioral responses to the visual stimuli. The experiment was not carried on longer than seventy days after recovery of vision.

Initially it was felt that the frog had great difficulty in inhibiting its snapping responses which suggests an inability to form associations involving feeding (Abbott, 1884). However evidence to the contrary has slowly been accumulated. Three species of Rana are said to have learned to reject a hairy caterpillar in four to seven trials with the memory lasting ten days (Schaeffer, 1911). Similarly, with a combination of aversive stimuli, electric shock and chemicals, a frog could learn to reject an earthworm in only two trials (Schaeffer, 1911). Razwilowska (1927) discovered that frogs could learn to eat only when a particular size square was presented along with the food, which is a complex feeding association.

One trial learning involving the rejection of perceived food has also been reported (Buytendijk, 1918a; Brower and

Brower, 1960). Moreover, Buytendijk (1918a) found that a European toad would reject red ants after one trial and then generalized the response to rejection of spiders and flies as well. The latter two insects subsequently during forgetting were eaten sooner than the red ants. Brower and Brower (1960) in their elaborate study of the evoluntion of mimicry between species found that Bufo terrestris would reject a robberfly mimic of the bumblebee after having taken only a single bee. These toads did not generalize their response to other insects, as did the frogs in Buytendijk's study, since they continued to take dragonflies as food. Moreover, stinger-less bumblebees were also rejected by the experienced toads. A normal feeding response to a particular species could be transformed into a defensive response after the toad had a noxious experience with that species (Brower and Brower, 1960). Potassium hydroxide can be used to train toads to reject food in one or two trials (Schmidt, Personal Communication).

Frogs and toads can also be induced to take a non-edible moving object, for example a piece of paper. The toad will subsequently refuse to eat another piece of paper for several minutes unless an insect is fed to the toad inbetween presentations of the placebo (Nobel, 1942).

Buytendijks' results indicated a short term memory in frogs. However, the results of Brower and Brower (1960) indicated a memory of seven days because dragon fly controls were readily

eaten while bumblebees and their mimics were constantly rejected.

Another evidence for the formation of feeding associati ons in anura comes from direct behavioral observation. Normally, toads will not orient towards someone approaching to feed them; however, with continued experience it has been reported that toads will learn to orient towards the caretaker (Nobel, 1942) and (Vandel, 1927).

A more objective investigation of the locomotor responses of anura in response to feeding was undertaken by Van Bergeijk (1967). Van Bergeijk maintained the animals in a social environment simulating their natural habitat. The bullfrogs were fed on a regular schedule. A camera mounted above the enclosure was used to periodically observe the distribution of animals within. A computer was used to analyse the data and to design a topographic map of the population density of animals with respect to time. The results indicated that the animals would consistently approach the feeding area slightly in advance of the regular feeding time. Van Bergeijk interpreted the results to mean that the frogs had learned both the time of feeding and secondly the location of feeding.

Certain findings of Van Bergeijk's investigation remain enigmatic. One enigma is the fact that the animals did not collect in the feeding area on days when they would not be fed, that is on holidays or weekend days. Even more puzzling is the fact that the frogs did not collect in the feeding area on other

days that were arbitrarily chosen by the investigator to be holidays.

A number of explanations are possible for Van Bergeijks' puzzling findings. One possibility is that the frogs followed one other frog and this was responsible for the observed behavior. Another explanation could be that the lights used for photography may have stimulated the animals' phototactic responses. The obvious explanation is that some stimulus in the laboratory was responsible for the collecting of the frogs in the feeding area. The results clearly indicate that the unknown stimulus was only present on the days during which the animals were fed. Since prey movements are such powerful activators of prey stalking behavior in the anura, it would seem that the prey were responsible for attracting the frogs to the feeding area. There is a possibility that the live prey, mealworms, were placed near the chamber shortly before feeding. Then the frogs could have noticed the food and began moving towards the feeding area prior to the actual feeding.

Van Bergeijk criticized the use of unnatural motivational stimuli such as shock for learning studies in anura and proposed that his investigational approach was more appropriate.

Although Van Bergeijks' results do indicate conditioning in anura, one can still be doubtful since Van Bergeijk hasn't objectively explained the frog's behavior on "holidays".

Recently a feeding hierarchy has been claimed to exist in

Rana pipiens and has been suggested as an example of learning in a social feeding environment (Boice, 1969).

An exciting area for investigation deals with the stability of learned responses acquired prior to metamorphosis and measured after metamorphosis. This area however has not been investigated very deeply. Such investigation is mentioned here since it relates to the study of the development of feeding associations in Amphibia. Some indication of whether changes could occur in learning acquired prior to metamorphosis is derived from the work of Haecker (1912) who found that feeding associations did not persist after metamorphosis in axolotls. Related to the above is the necessity to determine if younger amphibia could learn. Munn's work (1940) could give one some indication of an answer. Munn was unsuccessful in motivating tadpoles to learn a maze using food as a reward.

LEARNING EXPERIMENTS INVOLVING ELECTRIC SHOCK

The early history of the use of electric shock as an aversive stimulus in learning studies with frogs and toads closely parallels that of maze learning studies with these animals. The parallelism exists because the same investigators utilized both techniques (Yerkes, 1903a,b; Burnett, 1912; Munn, 1940). Most of the early investigations utilizing electric shock were unsuccessful in producing evidence of conditioning in Anura (Yerkes, 1903a,b,; Burnett, 1912; Munn, 1940). However, evidence of successful conditioning in Anura utilizing electric

shock has been relatively recent (Martof, 1962; Crawford and Langdon, 1966; Ray, 1967; Boice, 1970; Cann and Scudder, 1970).

A simple avoidance task involving a bright light as the CS and shock as the UCS was tried in Rana pipiens by Yerkes (1903a). The frog merely had to jump off the shocking grid in response to the light stimulus. No association was made between the light and the shock even after 180 to 450 trials (Yerkes, 1903a).

Yerkes observed that the frogs' movements were easily inhibited. The frogs' movements were inhibited by the strong conditioned stimulus light source and by the movements of the observer. Yerkes had chosen only animals of exceptional activity for use in his investigation. He felt that these animals were the healthiest and the easiest to condition. Yerkes suggested that the strong conditioned stimulus light source was responsible for his negative results. He hypothesized that the light source was too intense and acted to inhibit movement in the frog rather than to induce an avoidance response.

The results of the "reaction time" study indicated that frogs had extremely variable reaction times to a low voltage shock. Yerkes reported that the mean reaction times for the first one half of the trials was smaller than the mean reaction times for the last one half trials. Yerkes believed that the slower reaction times of the last one half of the experimental trials were not due to fatigue. Yerkes presented evidence which showed that the difference between the reaction times of the

last half of the trials and the first half trials was smaller when a greater stimulating voltage was used. Yerkes hypothesized that a higher voltage stimulus would have fatigued the animals more and thereby make them perform much slower in the later trials. Thus Yerkes' results showed that fatigue was not the causal factor in producing slower reaction times in the latter experimental trials.

Yerkes categorized the frog's reaction time values into three general classes: a very fast, an intermediate, and a very long. These relative terms apply to the amount of time the frog took to move its leg in response to the shock. The responses varied from either a mere twitch of the stimulated limb, or a combination of a twitch and a jump, to sudden jumps without a twitch.

Yerkes observed that these three classes of responses were associated with particular conditions and hypothesized about their genesis. The fast responses usually occurred after strong stimuli. The fast responses also occurred regularly in spinal animals. Therefore, Yerkes hypothesized that the fast responses were entirely due to spinal reflexes. The intermediate reaction times usually were associated with a weak stimulus. Yerkes hypothesized that the intermediate reaction times were a result of an interplay between spinal reflexes and instinctive reactions which utilized other parts of the central nervous system. The slowest reaction times weren't linked to a certain stimulus; he

considered these responses to be entirely volitional in nature (Yerkes, 1903b).

In conclusion, Yerkes claimed to have demonstrated learning in the animals of his reaction time study. However, only a few animals displayed changes in behavior which could be interpreted as learning.

Another investigation of learning in Amphibia which utilized electric shock as the unconditioned stimulus, had decerebrated frogs as subjects. Certain behavioral characteristics of decerebrate frogs were also reported in that investigation. Burnett (1912) observed that decerebrate frogs did not show much spontaneous movement, that is, they were relatively passive and sat for very long periods of time without moving. Therefore, it is not surprising that Burnett encountered some difficulty in getting his decerebrate animals to move through a maze; see maze learning section. To motivate the decerebrate frogs, he placed a drop of ether onto the skin of the frog at the start of the maze and applied electric shock to the feet of the frog farther into the maze. Burnett showed that the decerebrate frogs would jump blindly forward or attempted to climb out of the apparatus on receiving foot shock. Burnett found that the decerebrate frogs did not escape through the exit of the maze any faster with or without shock as a motivational influence (Burnett, 1912). In other words, Burnett found that electric shock applied to the feet of frogs did not induce or enchance learning.

Avoidance conditioning of Hyla versicolor tadpoles in a Y - maze was attempted by Munn (1940). The protocol involved the reversal of an innate positive phototropic response which made the tadpoles go to the more brightly lit section of the Y - maze. Electric shock was used as the aversive stimulus. Performance was quantified by determining the number of incorrect responses which occurred in ten trials. If a sufficient number of correct responses occurred by the 100th. trial, Munn interpreted this as a learned reversal in alley preference. The daily responses were quite variable and no seeming improvement occurred after the first one hundred trials.

Another similar experiment with Hyla cinerea tadpoles indicated improved performance with experience in a T - maze. In this case, electric shock was not used as the aversive stimulus (Munn, 1940). Light and vibration were used as the aversive stimuli. However, this species could not learn another T - maze in which the alleys were differentiated by vertical and horizontal stripes.

Munn also investigated the learning ability of Rana catesbeiana tadpoles in a four blind alley maze. Small lights illuminated the choice points in the maze and electric shock was used as an aversive stimulus. The number of incorrect choices were recorded for each choice point. A learning curve could be plotted in relation to the responses at the first choice point only. Munn concluded that the Rana catesbeiana tadpoles could

not learn the entire maze even after fifty days of training with five trials per day.

Munn modified his technique by removing the small lights at the choice points and decreasing the amount of electric shock administrated. This investigation also utilized Rana catesbeiana tadpoles. The data were recorded as mentioned above. The results indicated that no consistent learning could be observed except for the first choice point in the maze.

Munn concluded, from his experiments with shock motivated maze learning, that tadpoles were slow learners and that they do not show good retention even from day to day.

Bajandurow and Pegel (1932) reported that they conditioned an increase in buccopharyngeal respiratory rate and a general increase in motor activity in response to both visual and auditory stimuli. The species used was Rana esculenta. The authors found that if electric shock was used as a negative reinforcement then a sound or a colored light would produce changes in the respiratory rate and in motor activity. They also found that the animals responded more to auditory than to visual stimuli. However, when electric shock was omitted the responses extinguished in only two or three trials. Furthermore, Bajandurow and Pegel were unsuccessful when they tried to condition the frogs to differentiate sounds or colored lights. Bajandurow and Pegel concluded that they had conditioned their animals.

Capranica (1965) questioned the conclusion of Bajandurow and Pegel (1932). Capranica believed that the lack of evidence of discrimination between colored lights and sounds speaks strongly against conditioning in Bajandurow and Pegel's investigation. Furthermore, Capranica sites the fact that Bajandurow and Pegel were unsuccessful in demonstrating a conditioned response when the trials were presented too slowly, or when they waited until the next day. Capranica also pointed to the facts that the "conditioned" responses appeared very quickly after the shock and extinguished rapidly in the absence of shock. Therefore, Capranica suggested that Bajandurow and Pegel did not condition their animals but merely observed the phenomenon of sensitization.

Kleerekoper and Sibabin (1959) also investigated conditioning in Anura using auditory stimuli as the conditioned stimulus and electric shock as the unconditioned stimulus. They reported relatively poor results.

McGill (1960) used adult Rana pipiens in a shuttle avoidance procedure. Electric shock was used as the aversive stimulus. The fastest exit time was always the first trial of a naive animal. McGill found that with continued experience the exit time became longer. He found that the exit times became shorter than those of the preceding trials when a higher shock level was initiated. The exit time values then increased to beyond the previous values. McGill attributed the observed changes to

maladaptive learning since they led to the eventual death of some animals.

Martof (1962) offered positive evidence of conditioning in toads when he quite bluntly stated that he trained Bufo fowleri to hop onto a block to escape shock. Martof claimed that an avoidance response to shock could be conditioned by using an olfactory conditioned stimulus. Martof stated that toads avoided shock by moving onto the block, quite satisfactorily, after ten days of training with six trials per day. However, an anosmic animal could not learn this response. The main odorants were 2,2,4-trimethyl pentane, oil of sassafras, and an alcohol based perfume. The interval between the presentation of CS (odorant) and the UCS (shock) was ten seconds.

One must question Martof's report of successful avoidance conditioning in Bufo fowleri for several reasons. First of all, Martof did not physically represent his data in the literature. Secondly, the number of animals used was not mentioned; however, Martof did mention using one anosmic animal as a control. Additional doubt is raised by the fact that Martof did not represent a statistical analysis of the data. Furthermore, Martof did not even suggest that the responses were highly variable; an observation which has been well documented by other investigators (Yerkes, 1903a; Burnett, 1912; Munn, 1940).

More recently Ray (1967) studied avoidance conditioning in urodeles and found that electric shock was a poor stimulus for

avoidance conditioning since it usually produces an inhibition of motor activity. His observations in urodeles substantiates a similar observation made by Yerkes (1903a) in Anura. Ray subsequently found that either strong light or vibration was a better unconditioned stimulus than electric shock. Ray also achieved the avoidance conditioning of the urodele Ambystoma tigrinum using light and vibration (Ray, 1967 and Ray, 1970). Ray's detailed investigation presented strong evidence for the existence of learning in at least one species of Amphibia.

Up to now investigations utilizing aversive electric shock in the avoidance conditioning of Anura has yielded negative or questionable results. Therefore, up to now, one could find little evidence to support the hypothesis that learning exists in the Amphibia with this method. The following recent investigation utilized electric shock and its results support the aforementioned hypothesis (Boice, 1970).

Four species of Anura (Scaphiopus hammondi, Rana pipiens, Rana clamitans and Bufo woodhousei) were tested in a one-way avoidance conditioning procedure. The shock duration was limited to ten seconds. The safe box had either white or black walls. Two normally passive species Scaphiopus hammondi and Rana pipiens never avoided the electric shock. However, two relatively active species Rana clamitans and Bufo woodhousei produced reliable evidence of escape and avoidance. Rana pipiens escaped on an average from seven to ten seconds after CS onset. In Rana

clamitans these exit time values increased later on in the training sessions from less than two seconds to more than five seconds. Scaphiopus hammondi toads exited from the box about five seconds after CS onset. Bufo woodhousei toads were unique since their exit times showed a decrease with progressive experience. The starting escape time which was greater than five seconds decreased to less than one second. The Bufo woodhousei toads also avoided the shock 50% of the time after ten days of training of twenty trials each day (Boice, 1970).

Some of Boices' results are in accord with those of other investigators. For example, Boices' findings concerning the inability to condition Rana pipiens using electric shock agrees with the same findings by Yerkes (1903b) and McGill (1960). Furthermore, Boice partially substantiated McGill's (1960) finding that exit times tended to increase with progressive experience. The phenomenon is only partially substantiated since two different species, Rana clamitans in Boice's work and Rana pipiens in McGill's work, exhibited this result.

Since there is little evidence for learning in amphibia there also is little evidence to establish the duration of memory in amphibia. Although there is little evidence, a brief discussion about memory duration in amphibia shall be considered here. Yerkes (1903a) claimed a memory of greater than a month's duration in his maze learning studies. Brower and Brower (1960) also proposed at least a 7 day memory duration in their feeding

association study. However, the investigations which had successfully demonstrated learning in amphibia utilizing a shock avoidance procedure indicate a different duration of memory in amphibia than the studies which utilized either a maze or feeding association procedures. Boice (1970) found that the memory of previous experience was short lived in Bufo woodhousei since a performance deficit occurred after a two day interruption in training. Boices' results are in accord with the results of Crawford and Langedon (1966) who have stated that a daily relearning was necessary for the southern toad, Bufo terrestris, when being trained in a one-way shuttle avoidance procedure.

Some of the neuroanatomical correlates of avoidance in anuran behavior established by Ewert (1970) may lead to speculation about the pharmacology of anuran learning. Ewert determined that the pretectal area and the caudal thalamic region play a major role in the elicitation of avoidance behavior in the toad Bufo bufo. Toads with ablations of these areas failed to avoid those things which formerly elicited avoidance behavior. The avoidance behavior which was abolished by the lesions was the inhibition of movement. One must also remember that electric shock to the feet of anura also inhibited their movements. Since the suggestion has been made that pharmacological abolishment of the inflating reflex could possibly enhance avoidance conditioning in anura (Cann and Scudder, 1970), one might also speculate that drugs which affect the integrative actions of

neurons at the pretectal and caudal thalamic areas might also affect anuran learning.

OTHER INVESTIGATIONS RELATED TO ANURAN LEARNING

The long history of difficulty encountered in the conditioning of frogs and toads (Hempelmann, 1926; Karamyan, 1956; and Thorpe, 1956) has led to attempts at conditioning their reflexes. The eyelid blink reflex, corneal reflex, can be conditioned to a light poke on the nares (Goldstein et al., 1964). Goldstein indicated that a two second interval between the CS and UCS presentation was essential to the formation of an association.

The elicitation of wiping reflexes were transferred from one reflex genic zone to another (Franzisket, 1963). The transfer of this reflex activity was not due to a sensitization of periferal receptors (Franzisket, 1963). Franzisket's investigation (1963) was performed in spinal frogs. The results also implied that the frog's brain was not essential for the conditioning to occur.

Behavioral observations in the field have indicated that toads exhibit homing behavior. For example, toads will migrate long distances during the breeding season (Maynard, 1934, and Blair, 1943). Toads will also return to their point of origin (Jungfer, 1951) after being displaced of up to one quarter of a mile away. Young fowleri toads will leave and return to a shoreline using celestial cues for orientation (Ferguson, 1966).

Adult toads do the same sort of thing but are diverted from their courses by con-specific calls (Ferguson, 1966). Toads can also adjust their homing behavior to changes in the azimuth of the sun (Landreth and Ferguson, 1968). Such observations suggest that toads could be using certain environmental cues to learn their path about the environment.

If one assumes that learning is involved in the homing behavior of toads, then one would expect the toads to make associations between their environmental landmarks. A mechanism has been discovered by which a toad could use the sun as a landmark. Besides an optic mechanism, an extraoptic mechanism exists for celestial orientation and the entrainment of the toads biological clock (Taylor and Ferguson, 1970). The extraoptic mechanism is centrally located and is not associated with the dermal light sense in toads previously reported by Steven (1963). The extraoptic mechanism establishes an orientation towards the sun which is adjusted by the toads biological clock for the time of day. Presumably then an association is made between the toads orientation to the sun and some other aspect of the toads environment. Thus one could utilize the toads homing behavior to good advantage as a possible method for studying learning in toads.

BRIEF NATURAL HISTORY OF THE TOAD SPECIES USED IN THIS INVESTIGATION.

Four of the many toads that are indigenous to the United

States are: Bufo americanus, Bufo terrestris, Bufo fowleri and Bufo woodhousei. The major species studied in this thesis was Bufo fowleri. In addition the Bufo americanus species was also studied to some extent. The differences between these species of toads are subtle. Therefore, I shall relate the natural history of these four species as documented by Blair (1941).

The species are mostly genetically pure; however, they are naturally hybridized to some degree. The fact that local conditions may result in the formation of a number of subspecies is evidenced by a geographical cline which exists for the body-foot ratio in these four species. A larger ratio develops as one proceeds from northeast to southwest.

Certain physiological and behavioral characteristics tend to keep the species from interbreeding. For example, Bufo americanus breeds in April while Bufo fowleri breeds later on in May. Beside the different breeding dates the con-specific mating calls also tend to separate the species. In addition behavioral patterns also tend to separate the species. Bufo americanus prefers to breed in ditches and puddles of water while Bufo woodhousei likes to breed in more permanent bodies of water (Blair, 1941).

Certain characteristics serve to identify the species. Morphologically, Bufo americanus is characterized by ventral spotting, a tendency towards reddish coloration, being quite spiney, and possessing a longitudinal crest which connects its

reniform parotid glands with the post orbital crests. On the other hand, Bufo fowleri and Bufo woodhousei are relatively unspotted, are rarely red, are primarily green skinned, and are rarely "spiney". In contrast to Bufo americanus these two species have straight parotid glands and lack the longitudinal crest connecting the parotids to the post orbital crest. A difference in size distinguishes between Bufo fowleri and Bufo woodhousei. Few Bufo fowleri even attain the minimum size at which Bufo woodhousei begins to breed.

The natural ranges of the species differ. The different ranges may also function to maintain the uniqueness of each species. Bufo fowleri has a range primarily in the eastern United States. Bufo americanus has a range like Bufo fowleri but goes farther north and west but not quite as far south. The range of Bufo woodhousei is further west and only contacts the western limits of the ranges of both Bufo fowleri and Bufo americanus.

BEHAVIORAL CHARACTERISTICS OF ANURANS

Many environmental and physiological factors control the behavior of amphibia. The following paragraphs will document a few of these environmental and physiological factors. Among the environmental factors which will be discussed are light, temperature and seasonal variations. Among the physiological factors which will be discussed are hormonal states and autonomic tone.

Amphibia have very complex responses to light because they have at least three distinct light sensitive tissues or organs.

The amphibian eye is a functional organ which responds to object movements, object sizes and object shapes (Maturans et al, 1960) and (Ewert, 1970). The amphibian eye is important in feeding discriminations (Brower and Brower, 1960) and is partly involved in homing behavior (Landreth et al, 1968). The skin of amphibia is another light sensitive area. This dermal light sense controls phototactic responses (Steven, 1963; Parker, 1903; Torelle, 1903; Laurens, 1911; and Cole, 1922). In addition, some undefined extraoptic light sensitive area other than the skin, but which resides in the brain (persumably the pineal gland) is involved in celestial orientation and the entrainment of biological rhythms (Taylor and Ferguson, 1970).

Light can control the locomotor behavior of amphibia. Bufo fowleri will exhibit a positive phototaxis to either the larger or the brighter of two stimuli (Martof, 1962a). In addition, Martof (1962a) indicated a 0.94 positive correlation between the intensity of light and the proportion of toads which went towards it. The earlier researches of Riley (1913) also indicated a positive phototaxis related to light intensity. Rana virescens and Rana clamata also showed a positive phototaxis. Although these frogs stayed outside the perimeter of the lighted area they continually oriented themselves towards it (Torelle, 1903).

The work of Torelle (1903) indicated that the qualitative color properties of light could control amphibian behavior.

Torelle (1903) also found a strong positive phototaxis for blue light, a slight positive phototaxis for green, and a negative phototaxis for red light. Martof's (1962a) indicated that the quantitative properties of light could control amphibian behavior.

There is additional evidence which reinforces Torells's (1903) findings that the qualitative properties of light could control amphibian behavior. Martof provides some additional evidence by citing Dickerson (1906) who said that frogs could not distinguish between a lighted space and a white solid. Pearce (1910) demonstrated that toads exhibited a positive phototactic response towards the color blue. Boycott (1964) found that Hyla tadpoles have a dark preference. The conditioned reversal of phototactic responses has been used as evidence for learning in amphibia (Munn, 1940) and (Ray, 1967).

The ambient temperature is another environmental parameter which controls amphibian behavior. Martof (1962a) noted changes in the phototactic responses of Bufo fowleri to changes in ambient temperature. For example, at 9.7° C. most subjects stayed in the dark area. In other words at 9.7° C. most animals were negatively phototaxic. Neither a positive or negative phototaxis was observed at 14.2° C. since equal numbers of animals chose either the light or the dark areas. However, at 19.6° C. to 31° C. most subjects exhibited a positive phototaxis and chose the lighted areas. At the highest temperature tested,

37° C., the animals were again negatively phototactic.

In addition to observing the changes in Bufo fowleri's phototactic responses, Martof (1962a) also observed changes in the mode of locomotion which the toads used at the various tested ambient temperatures. At the lowest tested temperature the toads exhibited the maximum amount of climbing behavior. In addition, the toads utilized crawl-walking as a mode of locomotion at both high and low temperatures, and hopping as a mode of locomotion, at the intermediate temperatures.

Besides the environmental factors which have been discussed, there are physiological factors which control amphibian behavior. The altered physiological state of the animals is the primary change which results in the modification of behavior. One would expect that changes in neuronal activity could readily affect amphibian behavior. Indirect evidence exists which supports such a hypothesis. Chernetski (1964) reported that sympathetic stimulation could lead to a facilitation of Rana's responses to an auditory or tactile stimulus. One would also expect, that changes in the concentrations of putative transmitter substances might also affect behavior. Segura et al. (1967) have found that seasonal variations alter the catecholamine and hydroxyindoleamine concentration in the toad brain, they have related these seasonal variations in the biogenic amines to the hibernation - estivation cycle.

Seasonal variations could also induce altered physiological

states by producing hormonal changes in the animal. The altered hormonal levels might affect behavior profoundly. An example of a profound behavioral change is the induction of hibernation by seasonal variations (Nobel, 1942). A subtle behavioral change which occurs in response to seasonal variations and to alterations of hormone levels involves the mating behavior of frogs. Conspecific mate calling behavior can be initiated either by injection of whole frog pituitaires or by injections of chorionic gonadotrophin (Schmidt, 1966). Scant evidence has been accumulated which indicates that altered physiological states could affect learning behavior. Flower (1927) discovered that the hormonal changes which induce metamorphosis alter learning in the Amphibia. Haecker (1912) reported a learning deficit in the Amphibia during the breeding season. However, Munn (1940) found no difference between the performance of metamorphosed and non metamorphosed toads. From this brief survey of how altered physiological states might modify amphibian behavior, one might reasonably consider that hormonal changes could affect the learning behavior of amphibia. Thus, a few behavior altering hormones should be included for study in any investigation of drug induced modification of amphibian learning behavior.

BEHAVIORAL CHARACTERISTICS OF ANURANS RELEVANT TO LEARNING STUDIES

Knowledge of certain amphibian behavioral characteristics is of utmost importance in planning learning experiments. A

behavioral characteristic of importance in feeding association experiments involves the natural movements of food and their recognition by the frog or toad. One dogmatic principle of behavior which must be recognized is that frogs and toads only orient and snap at moving objects. Amphibians may starve to death if there is no movement to attract their attention (Yerkes, 1903). Amphibians will turn their heads and fixate on the approaching food. The orientation and intense fixation on its prey gives one the impression of intense concentration on the part of the frog or toad. With such an observation one might think that food as a positive reinforcement would be useful as a motivational influence in feeding experiments. Besides the complexities of having living and moving food; the size and movements of the food alter its motivational influence. Depending upon the size and rate of movement of dark objects,avoidance responses could be induced in toads (Ewert, 1970). These avoidance responses certainly can not be considered to be appropriate behavior for a "positive reinforcement". The only study investigating the use of food as a positive reinforcement involved a maze learning paradigm with tadpoles as subjects, and was unsuccessful (Munn, 1940). In the words of the investigators, "finding food of no use as an incentive, we made unsuccessful attempts to utilize escape from higher than normal temperature as an incentive" (Munn, 1940). One wonders if the aforementioned complexities of amphibian feeding behavior played

a role in Munn's failure.

Knowledge about amphibian avoidance responses is also useful in planning avoidance conditioning experiments. Toad avoidance responses have two basic patterns: hopping or leaping away, and freezing accompanied by squatting and inflation of the body. Only the hopping or leaping away type of avoidance response would be adaptive in the active avoidance conditioning procedures used in the present author's investigation. A previous investigator has found that fright and strong electric shock will inhibit movement in the anura. The animals will squat low and puff up and, may at times, touch their noses to the ground in a butting posture (Yerkes, 1903a). The inflating response occurs to noxious insects as well (Brower and Brower, 1960). However, frogs will jump away if the noxious stimulus comes from the rear (Yerkes, 1903a). Yerkes (1903a) reported that pain increased the escape time in an escape avoidance situation; however, he did not allude to which type of behavior was responsible for the observation.

Other behavioral characteristics of the Amphibia which should be considered in planning learning experiments are illustrated in the following experimental observations. Anura seem to be very stereotypic in their behavior. So much stereotypy exists that individual animals could be identified on the basis of their behavioral responses in the various training apparatuses (Yerkes, 1903a). Similarly, purposeless motor

routines are repeated in subsequent trials (Buytendijk, 1918b). In a reaction time study they showed a great uniformity of action but no signs of profiting from experience (Yerkes, 1903b). Furthermore, observations lead one to believe that frogs and toads will repeat their actions many many times especially, if it is not harmful or unpleasant (Yerkes, 1903a). Such stereotypic behavioral traits might make them difficult to train.

Decerebrate frogs show less spontaneous activity and eat voraciously without exhibiting an avoidance response towards the investigator (Burnett, 1912).

Toads also have distinct homing behavior utilizing a light mediated celestial orientation mechanism. Hormonal changes during the breeding season which stimulate innate calling can alter this homing behavior. Toads can also adjust their homing behavior with respect to the time of day which indicates that they have a sense of time (Landreth et al., 1968). Yerkes (1903) states that frogs are able to do a few things quickly and rapidly. However, they do not acquire new responses very readily. In summary, one must adequately plan in their experimental design for the behavioral traits of the Amphibia.

THE USE OF ELECTRIC SHOCK IN CONDITIONING TOADS

Some recent researchers have claimed that poor results in conditioning have resulted from the inability of other workers to properly motivate the animals (Van Bergeijk, 1967 and Boice, 1970). Similarly in studies with Ambystoma tigrinum strong light

and vibration have been found to be better unconditioned stimuli than electric shock (Ray, 1967 and 1970). Electric shock seems to encourage the passive avoidance reaction in frogs and toads, that is inflating and the inhibition of movement. The results of McGill (1960) and Kleerekoper et al, (1959) utilizing shock have been relatively unsuccessful and have preceeded the works of the critics. However, some of the earliest amphibian learning studies utilized electric shock with a good deal of success. The earlier investigations indicated some of the problems which were found using this method (Yerkes, 1903a; Burnett, 1912; Munn, 1940). There could be very little doubt that the initial experiences which Rana has with electric shock result in violent and frantic attempts to escape (Yerkes, 1903a; Burnett, 1912; McGill, 1960). Frequently, frogs and toads tend to persist in one direction of escape and fail in their ultimate attempt to escape (Yerkes, 1903a) and (Burnett, 1912). It is also well documented that the escape responses of Rana to electric shock decrease with progressive experience (Yerkes, 1903a, 1903b; McGill, 1960; Boice, 1970). It was also noted that the freshness of the animal was important in obtaining avoidance responses. Yerkes (1903b) also reported that cutaneous tactile stimuli seemed to lose their ability to initiate avoidance behavior even faster than electrical stimuli. McGill (1960) used the probability of injury from electric shock to account for the maladaptive response he observed.

In spite of these problems, there have been sufficient reports of successful conditioning in Anura utilizing electric shock that it cannot yet be considered a dead issue. Even one of the main critics for the use of electric shock in conditioning toads (Boice, 1970) was successful in using it. Munn (1940) was very successful in using both shock and tactile unconditioned stimuli to motivate tadpoles to chose one of two alleys in a Y maze. Martof (1962) briefly reported that Bufo fowleri could learn to hop onto a block to escape shock. Moreover, Martof (1962) showed in a related situation that an olfactory cue, which served as the CS, became associated with impending shock, the UCS. Two other groups, Bajandurow et al. (1932) and Crawford et al. (1966), also reported successful conditioning using electric shock as the UCS.

CONCLUSIONS OF EARLY RESEARCHERS

Yerkes (1903a) noted that inhibition of movement after strong stimulation is the usual case. Goldstein (1964) in introducing his study on reflexes cited Thorpe (1956), Karamyan (1956) and Hempelmann (1926) and concluded that frogs were difficult to condition because it was hard to restrain them and because they exhibited unstable responses. Sperry (1951) doubted that Amphibia could learn. Nobel (1942) stated, "The instinct patterns are so much more in evidence than learned behavior, that the latter type of behavior may well be neglected in considering the evolution of the groups."

Yerkes (1903a, 1903b) still the most astute observer of anuran behavior states that, "the green frog profits by experience very slowly as compared to most vertebrates." He further states, "the frog apparently examined its surroundings carefully, and just when the observer thinks it has made itself familiar with the situation it reacts in such a way as to prove beyond doubt the absence of all adaptation." Yerkes (1903a) can be relied upon finally to write the forword for this thesis in one sentence "Certain it is that one is safe in calling almost all the frogs' actions reflex or instinctive." This thesis along with other recent works such as those of Crawford et al. (1966), Ray (1967 and 1970) and Boice (1970) will refute the suggestion that one should neglect the concept of learned behavior in Amphibia.

METHODS

2-WAY AVOIDANCE CONDITIONING; THE CHOICE AND INTERFERRING FACTORS

This subsection of the thesis will concern itself with the author's experimental design. The following paragraphs will illustrate how the experimental design evolved around the behavioral characteristics of Anura. This section will also indicate which behavioral characteristics of Anura could have interferred with the conditioning procedure.

The foremost question about experimental design to be discussed is: Why use two-way avoidance conditioning to study learning in these animals? A number of factors were involved in choosing this approach. One factor was to use a training procedure in which the observer minimally handled the animals in between trials. Two-way shuttle avoidance seemed to be acceptable in this regard. Secondly, the familiar avoidance response of the frog or toad was a leap or a jump away from a noxious stimulus. Also this paradigm was well established as a procedure in mammalian studies where it had proved successful.

After having established two-way avoidance conditioning as the basic training method an aversive stimulus had to be chosen. Electric shock was used because it was readily controllable in intensity and because the reports in the literature suggested that it would be a useful procedure; see THE USE OF ELECTRIC SHOCK IN CONDITIONING TOADS, p. 35 above). Boice's results (1970) support the feasibility of the use of electric shock in avoidance training in the Amphibia.

One caution was obviously needed with regard to the use of electric shock as the UCS. The intensity of shock would have to be experimentally chosen to determine a useful value; see p. 57 METHODS SECTION). Once an effective shock level had been determined it became the standard UCS for all subjects.

Another complicating factor was the choice of a CS for the procedure. The choice of a CS depended heavily upon the limitations of the animals in perceiving the environment. The sense of vision was chosen because it was known to be functional. The bug detecting quality of anuran vision is well documented (Maturana et al., 1960). In addition Maturana et al. (1960) also demonstrated the presence of on-off receptors in the frog's visual system. These receptors responded to the presence or absence of light. Therefore, the lighting of a lamp was considered to be a detectable environmental change for the frog or toad. A white light was chosen as a CS to avoid any specific phototactic responses towards specific colors.

In the avoidance conditioning procedure it was necessary for the animals to perceive stationary objects in order to find the chamber exit. The work of Maturana et al. (1960) indicated that frogs had the ability to detect movement and the work of Brower et al. (1960) indicated that toads could distinguish shapes and patterns in moving insects. Although these two aforementioned works indicated a functional visual system, they gave no insight into the ability of the anura to visualize stationary objects.

Some evidence however exists to support the hypothesis that the anura can perceive stationary objects (Schipperheyn, 1963).

To control for the predominance of a positive or negative phototaxis influencing the subjects' responses to the CS, the following procedural design was followed. The animals had to either escape from a lighted chamber or into a lighted chamber. The results of Torelle (1903) and Boycott (1960) indicated a possibility of color preferences in these animals, the chamber walls were lined with colored construction paper. A light blue was chosen since this was supposed to induce a positive phototactic response (Torelle, 1903). Later a simple white-black preference was chosen since Martof (1962a) indicated that there was a positive phototaxis to lighted areas; see Appendix B and Methods, p.74). The temperature range of the testing was around 22°C. Martof indicated a positive phototaxis in the temperature range of 19.5°C. to 31°C. In addition, a control group was also put through the training procedure but with the CS light disconnected. This group, which served as a control, allowed the investigator to be sure that the animals were responding to the light rather than to sounds in the room or other environmental stimuli which were not under experimental control.

Some caution was necessary in the use of a light as a CS. The ambient background illumination could have easily interferred with the animals' responses. Therefore an experiment was designed to examine that possibility. The results (see p.165)

indicated that the ambient background illumination did affect the performance of the animals and therefore has to be controlled in an experiment of this type. When on, the CS light had to be of a different intensity than any other light within the chamber especially the photocell light. With regard to the spontaneous activity which was observed, the possibility exists that the dermal light sense could have been used to detect the photocell light source (Parker, 1903; Torelle, 1903; Laurens, 1911; and Cole, 1922).

Seasonal variations which readily change anuran chemistry and behavior (Segura, 1967 and Holzafel, 1937) could easily have affected their learning behavior. Unfortunately, the supply of animals also varied with the season such that they had to be purchased in advance and maintained under greenhouse conditions during times of inadequate supply, so a complete study of seasonal changes on learning could not have been undertaken. Furthermore, the fact that temperature affects the activity of these species requires that the temperature be kept within proper limits during the training. The room temperature ranged from 15.5°C.to 23.9°C. during testing depending mostly upon the season. The best learning occurred when storage conditions were strictly controlled and when the time of training was strictly controlled; see p. 165 . This reinforces the experiments of both Landreth et al. (1968) who also controlled temperature, humidity, and the time of training. Ray (1970) also strictly controlled the time

at which training occurred.

The inflating response of toads could have interferred with the experiments and it did; that is, the animals would inflate in response to shock rather than to actively avoid. However, with the choice of the optimum shocking level this was minimized. Besides inflation the normal passivity of the animals could easily make the avoidance conditioning a lengthy process. However, active avoidance conditioning demanded movement and thereby made the responses clear cut. It would be interesting to find out how much interference occurred between the normal behavioral passivity of the animals and passive avoidance conditioning.

The other behavioral characteristics which could have interferred with the conditioning were: homing behavior, fixated stereotypic and repetitive behavior. No attempt was made to control or test for interferences of the aforementioned variety. The repetitive behavior was a behavioral characteristic of the animals which was frequently observed during training. A control might be incorporated into future studies by reducing the size of the conditioning apparatus. Another approach is to design an apparatus where the animal is oriented in the proper direction for escape at all times such as a continuous alley maze.

GENERAL METHODS

Animals were purchased from the E. G. Steinhelber Company in Oshkosh, Wisconsin and from Tarpan Zoo in Florida. Bufo cognatus were purchased from the Pet Corral in Tucson, Arizona.

Subjects were adult male frogs and toads. It was impossible to determine the exact age of the animals therefore, their approximate dimensions are given below. On average the snout to anal distance was 10cm for frogs and 7.6cm for toads. The animals ranged in weight from 25 to 30gm. It takes about two to three years for the animals to become adults and attain this size.

The procedure for housing these animals has been developed by Schmidt and Hudson (1969) and was adopted for use during this research. Animals were kept in a greenhouse where the air temperature ranged from 14.4°C. to 37.7°C. in the summer and from 14.4°C. to 23.9°C. in the winter. Animals were housed in cement laundry sinks painted with epoxy paint and which were divided into dry and wet areas. The "wet area" contained a small quantity of water behind a ridge built into the rear of the sink. Fresh water continually dripped from the city supply into the "pond". Excess water was allowed to run off to a common drain. The animals were fed crickets twice a week from Selph's Cricket Ranch in Memphis, Tennessee. Occasionally, commercial Vionex powder (Squibb) was dusted over the crickets before feeding them to the toads.

In order to prevent contagious diseases from spreading throughout the toad colony, an antibiotic treatment period was instituted immediately after their arrival from the suppliers. Freshly received animals were placed in a soaking solution of oxytetracycline HCL. A concentration of 1 teaspoon per quart

of oxytetracycline was used. One half gallon of this solution was used in each sink and the solution was changed daily. Treatment continued for three to five days. Animals that arrived in poor health were reserved for experimentation until they appeared well. After the toads showed no interest in eating live crickets, a rapid decline in health occurred. It should be remarked that although many who were in poor health lived for months, their general condition did not improve. No attempts were made to force feed the animals since this procedure might have altered their general behavioral response towards handling. The species were kept in individual sinks. The sinks generally were not stocked with more than twenty Bufo fowleri, Bufo congatus, Bufo americanus or Rana pipiens and not more than ten Bufo marinus. Small animals were not chosen for training since they were not large enough to break the photocell beam. Therefore, experimental animals were approximately the same size and unhealthy and small animals were excluded.

Experimental subjects were removed from the greenhouse environment and transported in opaque plastic bags, as a group, to the laboratory where they were assigned numbers and stored individually in lucite boxes. The lucite boxes contained some tap water and were tilted to produce a dry as well as a wet surface. The relative humidity was around 100% because of a constant supply of water in the lucite box. During the experiment, animals were fed and their water was changed on a daily basis.

Experiments usually began two days following the transfer from the greenhouse. Empirically, it was noticed that the general activity of the animals was greatest on the day of transfer. Activity declined rapidly, however, so that approximately two days later the general level of activity was much less and remained stable thereafter. Behaviorally, toads seemed to sleep a lot, that is, not much spontaneous activity was seen while they were housed in the plastic boxes. However, periods of increased activity were also observed which seemed to be unrelated to any other laboratory activity. Direct handling, feeding, and changing of the water caused the toads to become very active. Occasionally, however, a fly would stimulate the animals into activity.

The procedure for changing water in the boxes involved moving the lid ajar and spilling out the soiled water, followed by rising twice with tepid water right from the tap. The animal remained in the container while the soiled water was changed. Crickets, as food, were also introduced into the containers after fresh water had been instilled.

On the days of experimentation, the container with the desired experimental animal was treated just as it was when the water was changed. The soiled water was spilled out while retaining the toad inside, and the container was rinsed twice with clean water. The subject was removed from the container and placed into the appropriate chamber. An animal always was placed

in the same starting chamber on every day of the experiment. To control for the influence of differences in the starting chambers, the animals were divided so that half of them started training in the left chamber and the other half started training in the right chamber. Thus a balanced design was created. When the animals were put in the starting chamber, they would always be placed far from the center with their snouts pointed in the appropriate direction for an avoidance response.

The training apparatus was designed and built in this laboratory since commercial types which were available did not seem suitable; especially, since their shocking grids were too widely spaced for the toads. However, the commercial grids were effective for rats and mice whose agile legs would not get caught between the bars. Figures 1 and 2 help to illustrate the basic structure of the apparatus. The overall outside dimensions of each chamber were 10" long by 8" high by 7" wide (a volume of 560 cubic inches). The shocking grid was made of round #10 guage busbar wires which were spaced three eights of an inch apart from center to center. The base, side walls and "common wall" were all electrified. The top was made of translucent plexiglass and contained a hole for a 6 watt,120 volt, conditioned,stimulus lamp. The lamps were centered and situated 2" before the common wall. The frame was made from eight plexiglass beams one half of an inch square and drilled to accommodate the wire. All wiring was in a vertical plane except the "common

wall" and base which were in the horizontal. A photoactive relay was placed between the chambers to record the crossing of the animals between chambers and to start the next trial. See Appendix A for the wiring schematic of the apparatus.

The major concept in design of the apparatus was to make the two compartments as similar as possible, so that the animal would be presented with essentially the same sensory input regardless of which chamber he was in. The walls were designed to carry shock and thus to prevent climbing in case those species with suction pads on their digits were used. In addition, it was considered imperative to guard against any bias from the investigator during the training sessions. Therefore, a two way shuttle avoidance procedure was chosen over one way shuttle avoidance. Thus, with two way shuttle avoidance the experimenter did not handle the animals between trials. Conceptually, the apparatus and the animal were considered to be a closed system, so that both would be allowed to interact with each other. Ultimately and hopefully, the toad would exhibit a characteristic and quantifiable behavior in this system which would result in predictable data. Meanwhile, the researcher was to observe and record the animal's behavior and serve as a master controller to make sure that the automated controls operated according to the planned experimental design.

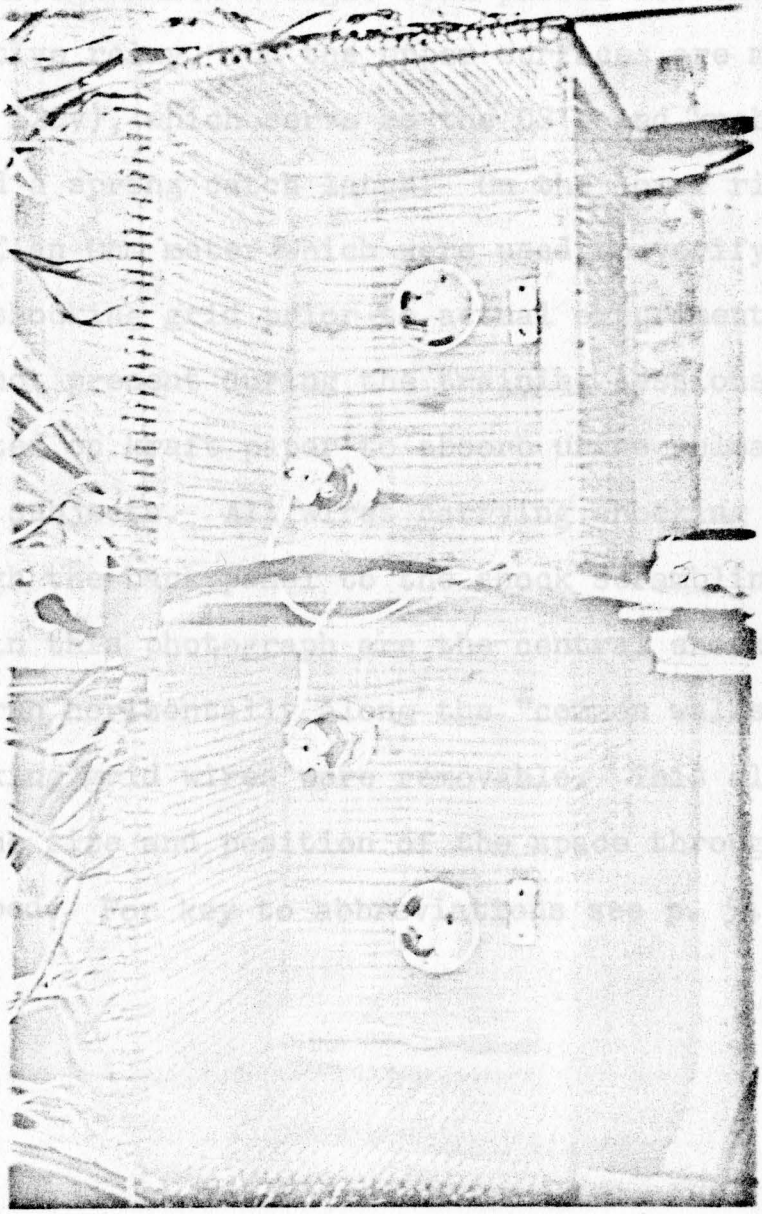


FIGURE 1

LEGEND FOR FIGURE 1.

The apparatus is divided into two compartments, left and right. The compartments are separated by approximately one half of an inch through which a light beam passes for the triggering of a photoactive relay. On the upper surfaces are mounted light sources (6w, 120v), which serve as the CS's and knobs to open the tops, and a spring catch latch. On the lower right are the two probes of an Ohm meter which were used to verify the integrity of the shocking grid prior to actual experimentation. These probes were not present during the training sessions. The chambers rested on kraft paper to absorb urine released by experimental subjects. All wires carrying shocking current are routed through the back panel to the shock scrambling device. Not evident in this photograph are the central shocking grid wires which run horizontally along the "common walls". The central shocking grid wires were removable. This allowed modifications in the size and position of the space through which the animals escaped. For key to abbreviations see p. 54.

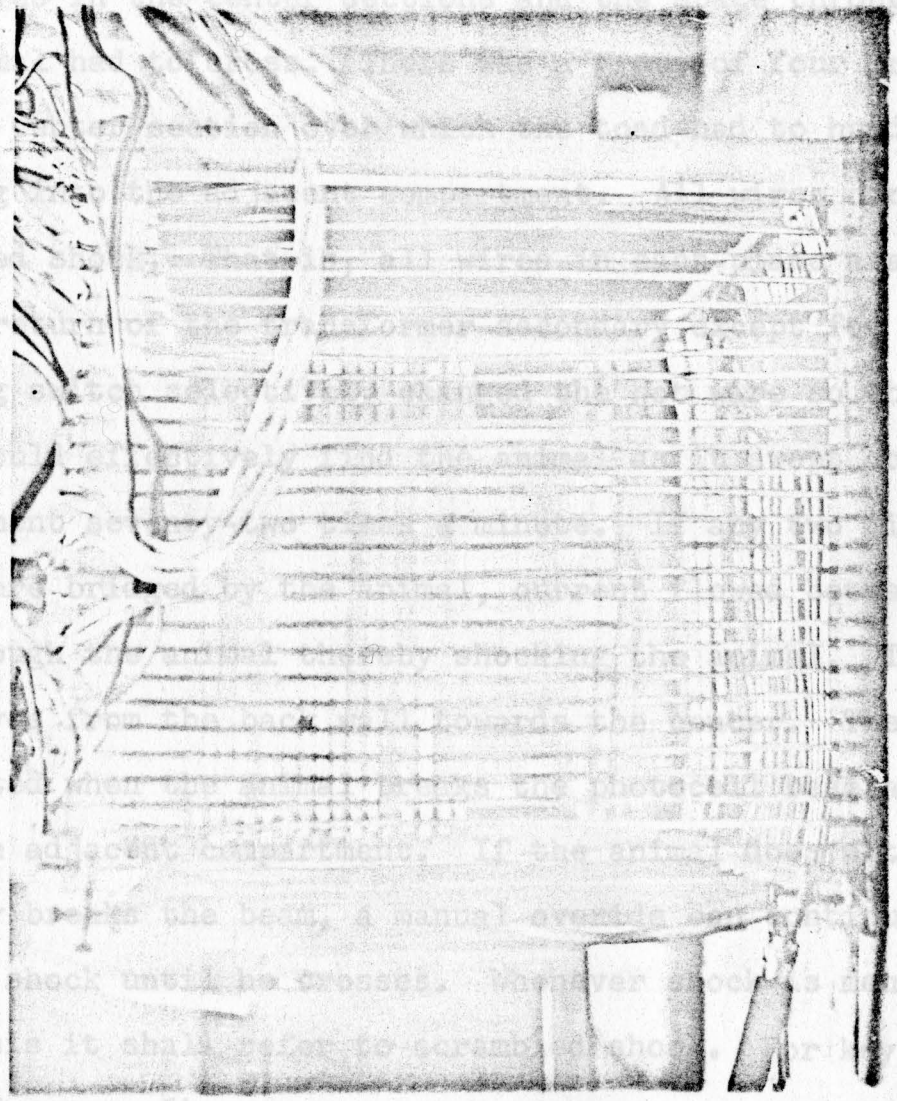


FIGURE 2

LEGEND FOR FIGURE 2.

This figure illustrates a toad, Bufo fowleri, in the right compartment of the conditioning apparatus. Note, the horizontal grid wires in the center sections and the space through which the animal had to cross. There was a group of four grid wires on each center section over which the toad had to hurdle while escaping into the adjacent compartment. All wires carried a scrambled shock. That is, all wires in each plane are "grounded" to the return of the transformer secondary except for one. A rotating switch selectively changed the hot wire so that the shock could effectively find the animal as it swept across the compartment seventy-two times a minute. If any two different wires were bridged by the animal, current flowed between them and through the animal thereby shocking the animal. The hot wire moves from the back wall towards the center. The shock is terminated when the animal breaks the photocell beam and crosses into the adjacent compartment. If the animal does not cross but only breaks the beam, a manual overide can continue the trial and the shock until he crosses. Whenever shock is mentioned in the thesis it shall refer to scrambled shock. For key to abbreviations see p. 54.

LIST OF ABBREVIATIONS FOR FIGURES 1 AND 2.

L	Left compartment (chamber)
R	Right compartment (chamber)
LS	Light source for photoactive relay
PAR	Photoactive relay
CS	Conditioned stimulus light
G	Shocking grid
W	Wires carrying shocking current
AP	Absorbent paper
TBP	Terminal board panel
MCS	Sites of modified CS used in later experiments
P	Temporary probes for monitoring shock

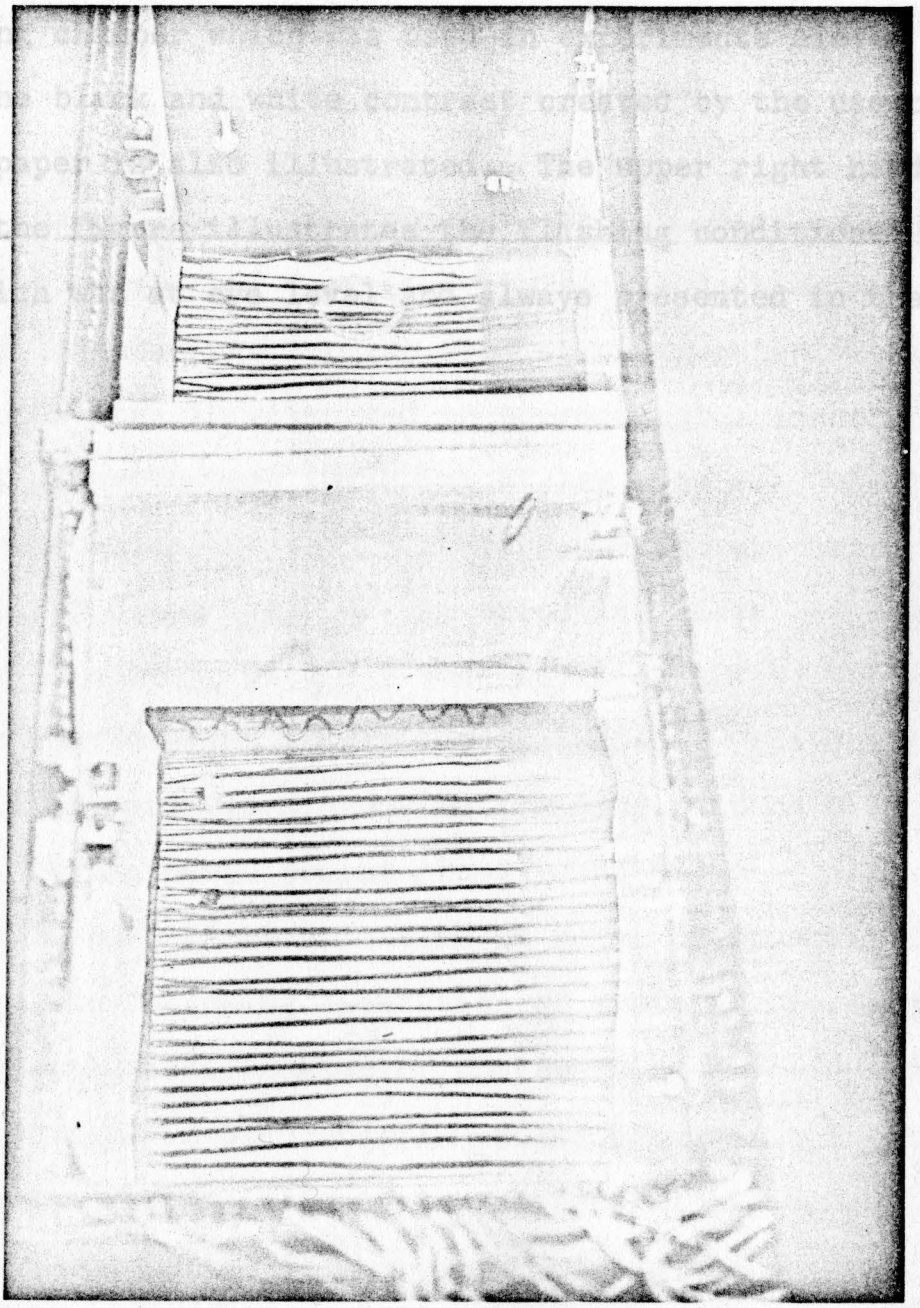


FIGURE 3

LEGEND FOR FIGURE 3.

This figure illustrates the final modification of the conditioning chamber which was used in experiments eleven and twelve. The black and white contrast created by the use of construction paper is also illustrated. The upper right hand corner of the figure illustrates the flashing conditioned stimulus cue which was at eye level and always presented in the safe chamber.

After construction of the apparatus an effective shock level had to be determined. Empirically, it was found that 4 volts was too low a level, since after only a few trials the animals were able to adapt to this level of shock. Evidence for this adaptation to the shock comes from their progressively longer exit times and also their refusal to leave the shocking chamber. Adapting toads crouched against the wires contacting more ground points and lessened the current flow at any particular point. A shock greater than 10 volts led to neuromuscular incoordination by producing involuntary twitching of leg muscles. The twitching muscles would incapacitate the animals and thereby impair the toads' ability to escape. A very effective shock level was found to be 7 volts. With a 7 volt level of shock, the animals neither became tolerant to nor incapacitated by the shock. In no case did any animals die during training. At no time did this shock level induce poison gland secretion.

It would be well at this time to refer to Appendix B in relation to the following discussion of training procedures.

In general, modification of the training procedure consisted of changes in the central hurdle; changes in the exterior of the shocking grids; changes in the central adjacent walls; changes in the interior, especially covering the shocking grid of the walls; modification in the type and position of the CS; changes in time intervals within the test trials, especially duration of CS, rest periods, etc. and changes in storage

environment of the animals. In Appendix B there is listed a table describing each training procedure. These changes in procedure presumably were responsible for the observed changes in behavior.

Originally it was decided that very few controls would be incorporated in the experimental design so that the design could be as simple as possible. Second, it was decided that if additional controls were required they would make themselves evident to the researcher. Therefore, the form which this thesis takes is one of a series of investigations using the trial and error approach to bring some insight to the solution of the larger question: Could these anura learn?

MISCELLANEOUS GENERAL METHODS, PROTOCOL AND DEFINITIONS

The following are standards developed for handling certain situations as they occurred:

A manual override switch was used to record a crossing between the chambers whenever the photocell failed to do so.

If the animal sat on the center and did not cross he was poked with a busbar wire which made him cross. Later a manual shock switch was incorporated and used instead of poking. This procedure was required infrequently.

No trial was allowed to exceed sixty seconds. If the animal did not cross by that time it was scored a mistrial and the apparatus was recycled to start a new trial.

If the photocell tripped more than once, a manual recycle made sure that the CS occurred in the appropriate chamber for the next trial.

If the CS appeared in the wrong chamber, the trial was scored a mistrial and a new trial began.

If the animal crossed into the safe chamber during the intertrial interval he automatically recycled the apparatus for a new trial; this was adaptive and scored a spontaneous crossing.

The chamber was not cleaned between animals except for large pieces of wet feces which might have shorted the wires.

Animals, because of the mode of storage and manner of starting the experiment, were wetted prior to being introduced into the chamber.

The chamber into which the animals were to escape was called the safe chamber.

MATHEMATICAL METHODS

Two types of information were basically recorded from these experiments: measurement data dealing with exit times, and enumeration data dealing with the frequency of spontaneous crossings. It is quite evident that each requires a different type of statistical analysis.

The following general guidelines were used in the data analysis. A mistrial was any exit time greater than sixty seconds, any trial where the conditioned stimulus went on in the wrong chamber, or any other occurrence which was not in accord

Basic Training Procedure and Characterization of Responses

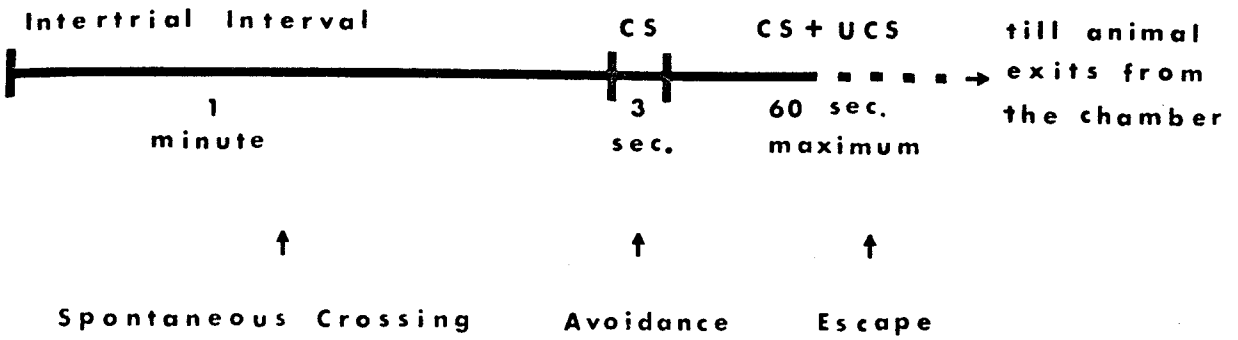


FIGURE 4. This figure depicts the terms spontaneous crossing, avoidance and escape as defined by when the animal moved from one chamber to the other.

with the experimental protocol. When a mistrial occurred one additional trial was added to the experimental session. Up to a point the number of daily trials in which the animal encountered shock was kept constant. An additional trial was also added to a session whenever a spontaneous crossing occurred. This additional trial was an attempt to give the animal the same quantity of shock daily. This was considered to be important since the electric shock was the motivating factor in this experiment, and it was considered to try to maintain a high level of motivation in day to day training. The total number of trials including mistrials, spontaneous crossings, and normal trials was set at a maximum of twenty per daily session. Twenty trials were chosen as the cutoff point, since it seemed that the animals would be fatiguing as their exit times increased. Mistrials were completely excluded from the calculations of mean exit times. However, mistrials were included in the calculations of the frequency of occurrence of spontaneous crossings only if the spontaneous crossing did not occur during the time period of the intertrial interval. Therefore, such a calculation would be a valid estimate of the frequency of occurrence of spontaneous crossing.

A common response in avoidance conditioning was that an animal who was learning escaped at a faster and faster rate until he avoided the aversive stimulus. Furthermore in classical responding, the percentage of trials in which the animal avoided

the noxious stimulus increased until it reached some arbitrary criteria designated by the researcher, or some asymptote. Learning curves took the form of plots of exit times versus experience or percent avoidance as percent spontaneous crossings versus experience.

In this thesis the following methods were chosen to analyse the measurement data. The exit times for a total day's trials were summed and averaged to give a mean exit time per day. The resultant plot was the mean daily exit time, the ordinate, versus the day of experiment, the abscissa. In addition, an average exit time was calculated for all trials bearing the same number. Thus, over the course of an experiment enough values were accumulated to indicate trends between trials. Learning was considered to have occurred when the faster exit times occurred in the later trials versus the earlier trials. In another approach, the mean exit times for the first half of all trials, trials one through five, were determined and compared to the last half trials, trials six through ten. Evidence for adaptive learning existed when the mean exit time for trials six through ten was lower than the mean exit time for trials one through five. Another gross indicator of learning utilized a comparison of the mean exit time between the first half of the experiment and the last half of the experiment. Similarly adaptive learning was said to have occurred when the mean exit for the last half of the experiment was significantly lower than the first.

With regard to spontaneous crossings, the enumeration type of data, the following analyses were made. At first, this researcher interpreted them as mistrials and did not record their occurrence other than by using the term mistrial. Then on the belief that these spontaneous crossings could actually be an adaptive response, they were recorded and analysed for changes in their relative frequency of occurrence.

Some major assumptions have been made with regard to the handling of the data. It had been assumed that an individual animal would respond to a stimulus in his environment the same way every time he encountered it. This assumption precludes phenomena such as learning, sensitization and habituation all of which play a role in the total behavior of an organism. However, to determine the influence of these phenomena one must first assume that they have no influence in the procedure. Therefore, an animal is depicted as an automaton, a mechanistic system, which if it has a variety of responses will continue to repeat that same variety of responses to the stimuli of his environment. This assumption allows one to make trial to trial comparisons of an individual animal's responses. One must also assume that the variety of behavior which was seen in response to the procedure yielded a reproducible variety of exit times. If so, then the exit times could be used to described those behavioral responses. Thus the measurement of the total behavior of the animal in this system was reduced to a measurement of his exit time or spontaneous

activity. Statistically speaking then, if a sufficient number of measurements have been made, the distribution of any group of observations should not be significantly different from any other group of observations.

Since increases in the speed of performance, that is decreased exit times, are observed when learning occurs one can choose for comparison those groups of observations which most likely would show such changes if learning were occurring. Therefore, I have chosen the exit times of the first five trials and have compared them to exit times for the last five trials. A paired student "t" test is used to test the hypothesis that both populations of observations are of the same population. If the "t" statistic indicated a significant difference between these two means, at a 5% probability of it being due to chance alone, and if the exit times for the later trials were lower on average than for the first trials, then the difference was said to have been due to adaptive learning. The paired "t" statistic was only used for intra-group comparisons. However, one cannot assume such reproducibility of behavior when inter group comparisons are made. Therefore, the unpaired "t" statistic was used for the inter group comparisons of measurement (exit time) data.

A basic assumption to the use of the "t" statistic is that the data are normally distributed. To test whether these data could be considered to be normally distributed two nonparametric statistical tests were used. The Wilkoxan Rank Sample Test and

the Sign Test are two nonparametric statistical tests that do not need the assumption of normality of data to be valid. Differences found to be statistically significant with these tests were also found to be statistically significant with the standard student "t" tests. Therefore the student "t" statistic has been used throughout. Because of the wide variation seen in the exit time values, linear regression analysis could not be used to predict how the exit time values would change with respect to trial number. The Pearson Correlation coefficients were 0.2 to 0.4 indicating very little if any correlation.

The enumeration data are the spontaneous crossings. These data are binomially distributed. That is, one is dealing with occurrence and non-occurrence data. Consequently the appropriate statistical tests have been used for these types of data. The Chi Square statistic and the binomial "t" test are applicable in these cases. These tests were used to quantify the probability of error in the observed differences in the frequency of occurrence of spontaneous activity. Corrections for "class" size and in some cases the exact probabilities were determined when the Chi Square test was used (Batson, 1960). Intra and inter group comparisons were made. Inter group comparisons of the frequency of occurrence of spontaneous crossings for the entire experiment were made. Intra group comparisons involved day to day and trial to trial analysis. If the value of Chi Square statistic exceeded that which would be observed 5% of the time due to chance alone,

and if the frequency of occurrence of spontaneous crossing was greater in the experimental versus the control group, then the difference was considered to be statistically significant and due to the training procedure. Likewise, if the former was again true and the frequency of spontaneous crossing was greater at the end of training with respect to trials or days, the difference was again considered to be statistically significant and due to learning.

The measurement data are represented in graphs of exit times (ordinate)per training unit (abscissa). The enumeration data are reported as tables and histograms of the frequency of occurrence of spontaneous crossing (ordinate) versus the training unit (abscissa).

SPECIFIC EXPERIMENTAL PROTOCOLS

Experiment 1. The species studied was Bufo fowleri. One animal was on a test/train/test paradigm. Trials one through five and sixteen through twenty were called test trials. On the other hand, trials six through fifteen were called training trial.

In a test trial the CS, an overhead light, was on for six seconds in the shocking chamber and followed by five seconds of darkness; following this electric shock continued until the animal exited from the chamber.

A training trial had both the CS, an overhead light, and the electric shock occur simultaneously in the shocking chamber, both continued until the animal escaped.

A basic description of the apparatus and ambient conditioning follows. The shocking grids were as depicted in Figures 1 and 2. Obviously, everything outside of the chamber was clearly visible to the animal since the wires were uncovered. The fluorescent room lights were also on. The candelabra holding the light bulb was covered with a clear lens cap.

A five minute rest period preceded each major switch from testing to training and from training to testing trials. The intertrial interval was 1 minute. Avoidance occurred if the animal crossed in eleven seconds or less after the onset of the CS, the overhead lamp.

The hypothesis for designing this type of procedure was that the first test trials of every day would test the retention of learning from previous experience; while the last test trials would be a measure of either fatigue or the best level of performance at the end of the current day's training. It was also hypothesized that the real period for forming the association between the CS and the UCS occurred during the so-called "training" trials. The hypothesis borrowed from the idea that the training trials provided a greater environmental impact upon the animal because both the CS and UCS occurred simultaneously. On the other hand, "testing" trials were thought to be a measure of the degree of this association.

The five second dark period, in the testing trials, was considered to be of value since the change in illumination

defined an obvious and hopefully an easily detectable time period for the toad. It gave him the time to process the sensory input generated by the flash of light and to respond. Theoretically, if there had been an association formed between the light and the shock the animals should have been stirred into avoidance by the six seconds of light alone, while the dark period gave them an extra five seconds to avoid being shocked.

The training procedure was designed partially in the classical Pavlovian sense to aid the formation of an association between the light (CS) and the aversive shock (UCS). The Pavlovian design involved the training trials, trials six thru fifteen inclusive, where both the CS and UCS occurred simultaneously.

Besides Bufo fowleri 1 (BF_1), BF_2 underwent the same procedure but without rest periods; a third, BF_3, underwent training trials only.

Coincidentally to the training of Bufo fowleri, single members of other species were also trained. A Bufo marinus (BM) was put through the test/train/test paradigm without rest. A Rana pipiens (RP) was put through a training only paradigm. Subsequently, the realization that learning could occur during the "testing" trials led to the modification of this test/train/test procedure.

Experiment 2. This was the first revision in the training apparatus. The space between the center walls was made opaque

with a sheet of white construction paper. It was felt that this created a unique area in the chambers that the toads could more easily locate. In addition, the glass of the room door was opaqued to prevent activity in the hall from interfering with the training procedures.

The same three Bufo fowleri, BF_1, BF_2 and BF_3, of experiment 1 were used. These animals were used because it was felt that they had, by now, become familiar with the chamber and thereby would more readily locate the escape route. It was also hypothesized that these animals having had experience in avoidance conditioning would show evidence of learning sooner than naive animals. All other parameters were kept the same.

Experiment 3. In this procedure a major change was instituted. The trials which tested for an association between the CS and UCS, the test trials, were abandoned since they produced an inconsistent environment and were thought to be responsible for the variability seen in the exit times of the preceding experiments. It was felt that more consistent day to day measures of learning and retention could be made by using all training trials instead of mixing in the so-called "test trials". Therefore a pure avoidance conditioning procedure was used. The duration of CS appearance was decreased to two seconds. This was followed with both the CS and the UCS until the animal escaped. The subject was a Bufo americanus (BA).

Meanwhile another Bufo americanus was undergoing the same

procedure, but with a red light as the CS in one chamber and a blue light as the CS in the other chamber. The objective was to determine if an innate, color, specific, negative, phototaxis would serve as a better CS than the white light. One may wonder if color vision is a possibility in these animals, since there is some suggestive evidence for it. Specifically, Liebman and Entine (1968) have identified various visual pigments in the frog's retina. In addition, Torelle (1903) has observed color preferences in the frog. Thus some scientific observations supported the use of different colored lights as conditioned stimuli in this procedure.

Experiment 4. This experiment was the first employing the species Rana pipiens. The new training procedure was followed. In contrast to experiment three, only one color was chosen for the conditioned stimulus. A white light was used as the conditioned stimulus in both chambers.

Experiment 5. Rana pipiens was the species used in this experiment. The animals, however, were limited to a maximum of sixty seconds of shock per trial. This design was chosen in an attempt to circumvent the unsatisfactory results reported by McGill (1960). A similar shock limiting procedure was used by Boice (1970).

Experiment 6. Intuitively it was felt that, in some procedures, having the room lights on improved the performance of the animals. Therefore in this experiment there were two groups of

animals. One group was trained with the room lights on while the other group was trained with the room lights off.

Characteristically frogs and toads jump into nearby ponds to avoid danger. The anura also have a positive phototaxis towards blue lighting (Pearce, 1910 and Boycott, 1964). Thus blue construction paper was placed on the outside of the far walls of both chambers. The blue paper supposedly resembled a patch of water and thus acted as an orienting device for the toads. In addition, the outer side walls were covered with black construction paper. The black construction paper prevented things which were occurring outside of the conditioning chamber from distracting and interfering with the conditioning process.

The inside wires were still exposed on all walls. Moreover, the bottom wires of the center section which formally served as a hurdle were removed. Thus escaping was simplified to the crossing of a dividing line rather than the climbing of an electrified hurdle. The CS appeared overhead in all cases in the safe chamber for three seconds prior to shock onset. The intertrial interval was one minute. The shocking voltage was 7 volts. The CS appeared for three seconds and then was followed by CS and UCS till escape.

Ten trials were given per daily session and spontaneous crossings were recorded. The design required a minimum of ten trials to be given when the animal did not avoid the shock. However, twenty trials per day was set as the maximum when the

animal did avoid shock. If a mistrial occurred, an additional trial would be added to the original ten to replace the bad trial. If a spontaneous crossing occurred, another trial would be added in an attempt to keep the number of shocked trials to ten. Therefore the total number of mistrials, spontaneous trials, escape trials, and avoidance trials numbered a minimum of ten per day to a maximum of twenty per day. Training continued for five days.

Experiment 7. Designed to see if a larger number of training trials would be successful in yielding better performance. The room lights were off during training. Fifty trials per day with the overhead CS light appearing in the shocking chamber were given to BF_6 while sixty trials with the overhead CS light appearing in the safe chamber were given to BF_{11}. In both cases the inside wires were exposed. Black construction paper lined the exterior of the side walls and the base while the far walls were lined with blue.

Experiment 8. Designed to indicate a difference between an overhead CS, no CS, and a flashing CS. All CS's appeared in the safe chamber. BF_{12-16} had an overhead CS while BF_{17-21} had no CS, and BF_{22-26} had a flashing CS at a rate of twice a second. The intertrial interval was one minute. CS duration was three seconds and shock was limited to a maximum of sixty seconds; see Figure 4 . The CS and UCS followed CS alone and continued until escape. Avoidance was an exit time less than or equal to

three seconds. All inside wires were exposed. Black construction paper lined the outside of the base and side walls. The far walls were lined on the outside with blue construction paper. Room lights were off in all cases and the animals received from ten to twenty trials per day for five days.

Experiment 9. Since much climbing of the inside walls was observed in all previous training, it was decided to line the inside wires with construction paper. The base or floor grid was left uncovered and became the only shocking surface. It was hoped that this would effectively eliminate climbing behavior and produce faster exit times by removing an opportunity to waste time in a maladaptive behavioral pattern. Therefore the inside center and sides were lined with black and the inside far walls with blue construction paper.

Besides Bufo fowleri another species Bufo americanus (BA) was trained since they seemed to perform better in previous procedures. Room lights were off during training. BF_{27-29} and BA_{20-21} received the CS overhead in the safe chamber. BF_{30-32} and BA_{22-23} received no CS. Meanwhile BF_{33-35} and BA_{24-25} received the flashing CS in the safe chamber. The intertrial interval was one minute. The CS and the control, no CS, durations were three seconds. Avoidance could only occur during these three seconds. Ten to twenty trials were given per day. Training continued for five days.

Experiment 10. The preceding experiment was repeated with

Bufo cognatus (BC) which seemed to be overtly more active, alert and quicker than either Bufo fowleri or Bufo americanus. When they all were present to compete for food in a sink, Bufo cognatus was first to capture its prey followed by Bufo americanus and then by Bufo fowleri. BC_{7-9} had the overhead CS in the safe chamber while BC_{4-6} had no CS, and BC_{1-3} had a flashing CS in the safe chamber. CS duration was three seconds and the intertrial interval was one minute.

Experiment 11. The study was taken now to its next to last modification. The simplest and most provocative contrast was chosen to replace the black and blue decor of the interior chamber. The new decor consisted of lining the entire inside of the chamber with white construction paper except for the far walls which were lined with black. Thus a black and white contrast was used for these and the following experiments. The room lights were turned off and the overhead CS in the shocking chamber was reinstituted since it would have greater impact in the white enclosure. BF_{36-40} had the overhead CS presented in the shocking chamber. BF_{41-45} had no CS presented and BF_{46-50} had the CS presented overhead in the safe chamber. Again the CS duration was three seconds and the intertrial interval was one minute. Trials ranged from a minimum of ten to a maximum of twenty per day for a period of five days.

Experiment 12. This was the final modification of procedure made in the study of avoidance conditioning. It involved a

controlled storage environment, staggered training times as a control for diurnal rhythms, controls for room lights, and controls for cues other than the CS cue in addition to all prior controls.

Animals were housed in individual plastic refrigerator containers with net tops and stored in a Percival environmental cabinet. Fifteen animals were investigated. Lighting was a mixture of 75 watt incandescent, and 40 watt fluorescent day light and 40 watt fluorescent "vita light" bulbs. The animals were at a distance of 1 foot from the fluorescent tubes. A daily rotation within the cabinet prevented each animal from receiving only one type of illumination. Animals had their water changed and were fed three to five crickets daily. In addition, a rubber stopper was placed in the container to serve as a dry surface. The animals were trained for sixteen consecutive days. Results were tabulated for the entire experiment and divided into groups of five days.

RESULTS

In this section of the thesis, the results are presented for all of the experiments that were discussed in the Methods Section. The reader should note the cross references to Appendix B and the Methods Section of this thesis. These cross references will help the reader to understand the changes in procedures from experiment to experiment and to relate the changes to the observed results.

The results of experiment one are depicted in Figures 5 and 6; see Methods Section p. 66 and experiment 1 Appendix B. The average exit time values for the first test trials differed from the last test trials and the training trials. The average exit times for the first test trials were reciprocally related to those of the last test trials and the training trials. Thus, whenever the first test trials resulted in a relatively long average exit time, both the subsequent training trials and the last test trials resulted in relatively short average exit times. In order to quantify this observation a linear regression analysis was performed on the data. The regression lines are illustrated in Figure 5. The abscissa in both cases is the average daily exit time for the first test trials. The left side of Figure 5 depicts a negative linear correlation between the first test trials and the training trials with a Pearson Correlation coefficient of -0.85. The right half of Figure 5 depicts a negative linear correlation between the first test trials and the last test trials with a Pearson Correlation

LINEAR CORRELATION OF DAILY PERFORMANCE BY TRIAL BLOCKS FOR BF 1

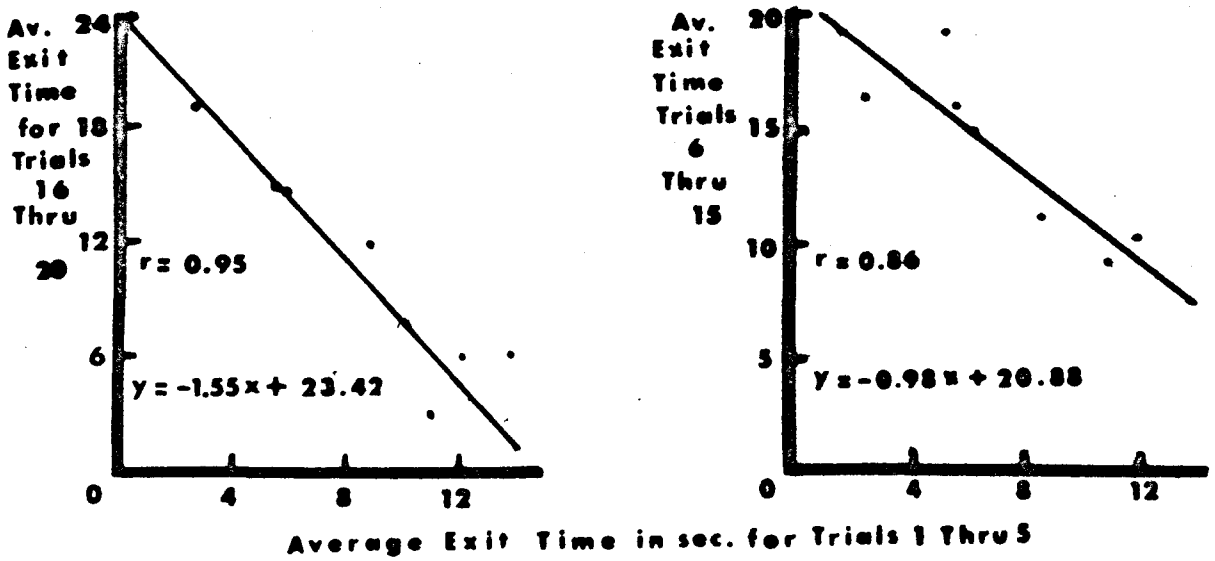


FIGURE 5

LEGEND FOR FIGURE 5.

This figure indicates the strong negative correlation between the average values for trials one thru five with both the values of trials six thru fifteen and trials sixteen thru twenty. One day without training occurred between the last two days on this procedure. The training procedure was a test/train/test paradigm; see Methods, experiment one, p.66. The graphs on the right indicate the relation of trials one thru five with the training trials (trials six thru fifteen); the Pearson Correlation Coefficient was -0.86. The graph in the lower left indicates the relation of trials one thru five with the last test trials (trials sixteen thru twenty); the Pearson Correlation Coefficient was -0.95. The graphs are of the linear regression line with the actual experimental values plotted alongside.

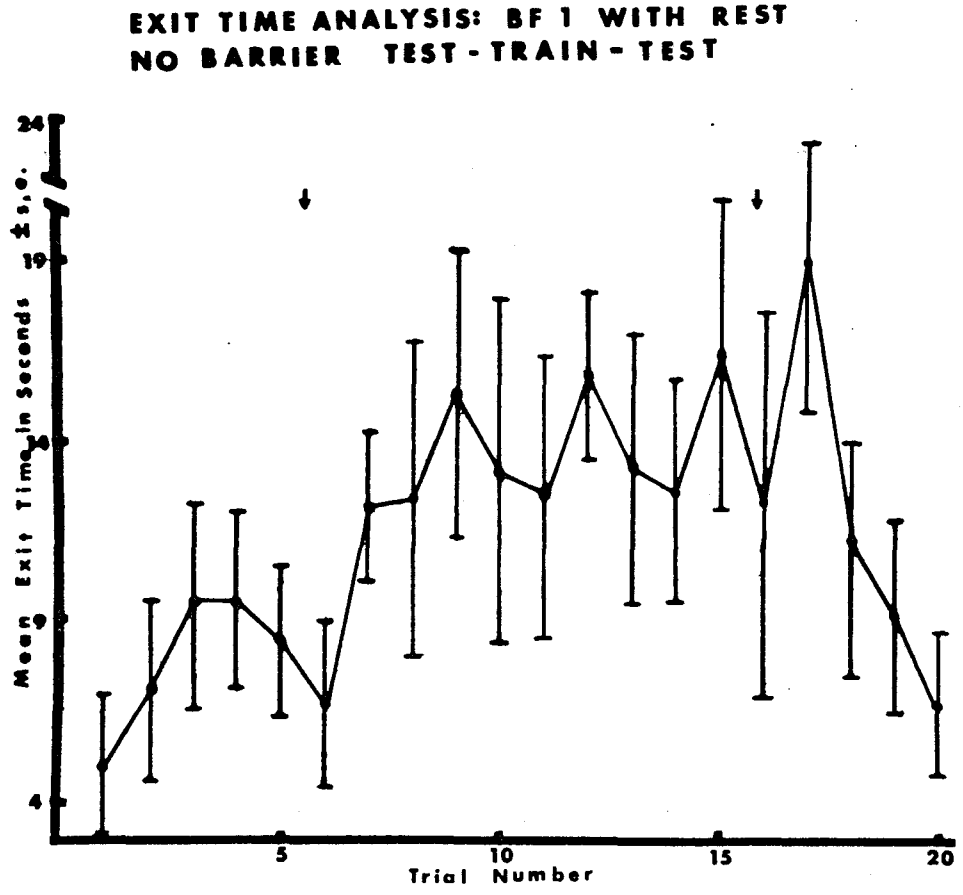

EXIT TIME ANALYSIS: BF 1 WITH REST
NO BARRIER TEST - TRAIN - TEST
Mean Exit Time in Seconds ± s.e.
24
19
14
9
4
5
10
15
20
Trial Number

FIGURE 6

LEGEND FOR FIGURE 6.

The graph depicts the mean exit time ± S. E. calculated for the same trial number of every experimental session (ordinate). The trial number is plotted as the abscissa. Trials one thru five and trials sixteen thru twenty are "test" trials, while trials six thru fifteen are training trials. Results are for one animal with eight continuous days of training. No attempt was made to train the animal at the same time each day. A three minute rest period occurred between trials five and six and trials fifteen and sixteen which is indicated by the arrows. The lowest average exit time occurred on trial one. The first trials following the rest periods also had relatively low values. There seemed to be a general trend towards longer exit times from trials one thru seventeen after which shorter exit times occurred. Trial seventeen also exhibits the highest average exit time value.

Coefficient of -0.95. Correlation coefficients of close to 1.0 are considered to be statistically significant.

However, Figure 6, a plot of average exit times of the same daily trial versus the corresponding trial numbers, shows a remarkable difference between the animal's performance during a test trial and a training trial. The lowest values are for trials one and twenty. Meanwhile other minimums occur especially after rest periods; see trials one, six and sixteen. Furthermore, on the second trial following a rest period the values begin to climb again; see trials two, seven, and seventeen. One biologically important finding was the general tendency for exit times to increase. This finding recalls to mind the similar finding of McGill (1960). The pronounced decreases in the average exit times for the last test trials suggest a certain amount of conditioning in this animal. In contrast to the results of McGill (1960), it was not necessary to increase the shocking voltage to maintain performance in this animal.

Unfortunately however, not a single exit time approached the level of avoidance nor did it even go below the initial value. This observation remained consistent throughout the rest of the experiments.

When an opaque barrier was placed between the wires of the center, a slight change occurred; see Figure 7. The exit times during the "training" part of the procedure seemed to level out since there was no steady trend towards higher exit times. This

animal still displayed low exit time values after rest periods, note trials six and sixteen. This animal also showed higher values on the second post rest period trial; note trials three, seven, and seventeen. Once again trials one and twenty had the lowest values, while trial seventeen again showed the highest exit time. The last four trials once again displayed a consistent trend towards lower values, possibly some avoidance or adaptation was occurring.

Similar to BF_1 another animal, BF_2, underwent the same training, but without rest periods. Figure 8 depicts the average exit time for each trial during the experiment. In this case the picture is slightly different from the case of BF_1. Both trials one and twenty are no longer the lowest. Moreover, trials six and sixteen do dip but not as much as the dips that were so obvious for BF_1.

One difference between the responses of BF_1 and BF_2 which might be attributed to either individual variation or procedural variation concerns the last four data points observed for BF_2. These showed a dramatic increase which was the complete opposite of what had been observed previously for BF_1. Once again, trials one thru five, the first testing trials, exhibited relatively lower average exit time values.

Figure 9 depicts the behavior of BF_2 after the addition of a white opaque paper barrier between the center grids. Unfortunately, the belief that this would stabilize performance was

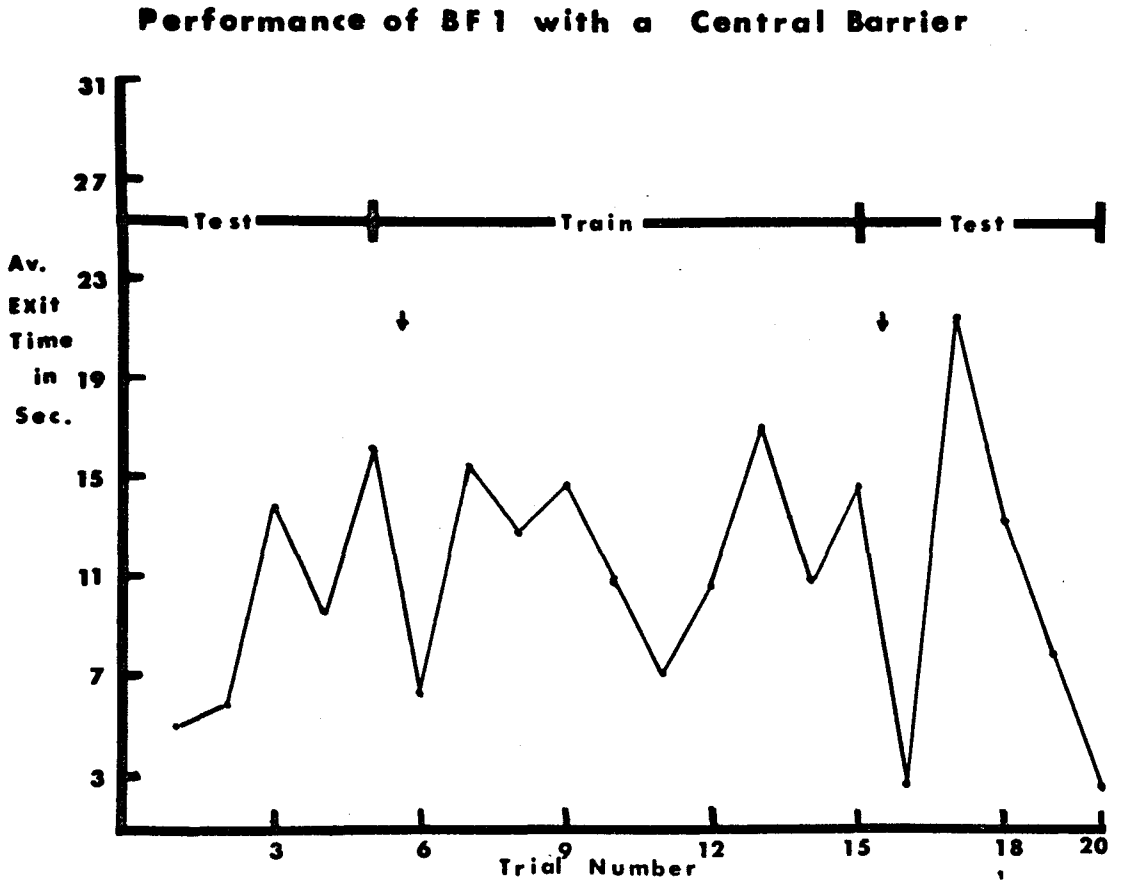

Performance of BF1 with a Central Barrier
Test
Train
Test
Av. Exit Time in Sec.
31
27
23
19
15
11
7
3
3
6
9
12
15
18
20
Trial Number

FIGURE 7

LEGEND FOR FIGURE 7.

In this graph the mean exit time calculated for the same trail number of every experimental session is plotted as the ordinate. The trial number is plotted as the abscissa. Trials one thru five and sixteen thru twenty are "test" trials, while trials six thru fifteen are training trials. Results are for one animal with four days of training. No attempt was made to train the animal at the same time each day. A three minute rest period occurred between trials five and six and trials fifteen and sixteen. These are indicated by the arrows. An opaque piece of white construction paper was placed between the "common" walls of the chambers to add more contrast. This would allow the escape route to be found more readily. Note, that the lowest values are for the first test trials and the trials immediately following the rest periods, trials six and sixteen. Also trial seventeen shows the highest value as in Figure 6. Note, also an apparent improvement in performance in the last three trials.

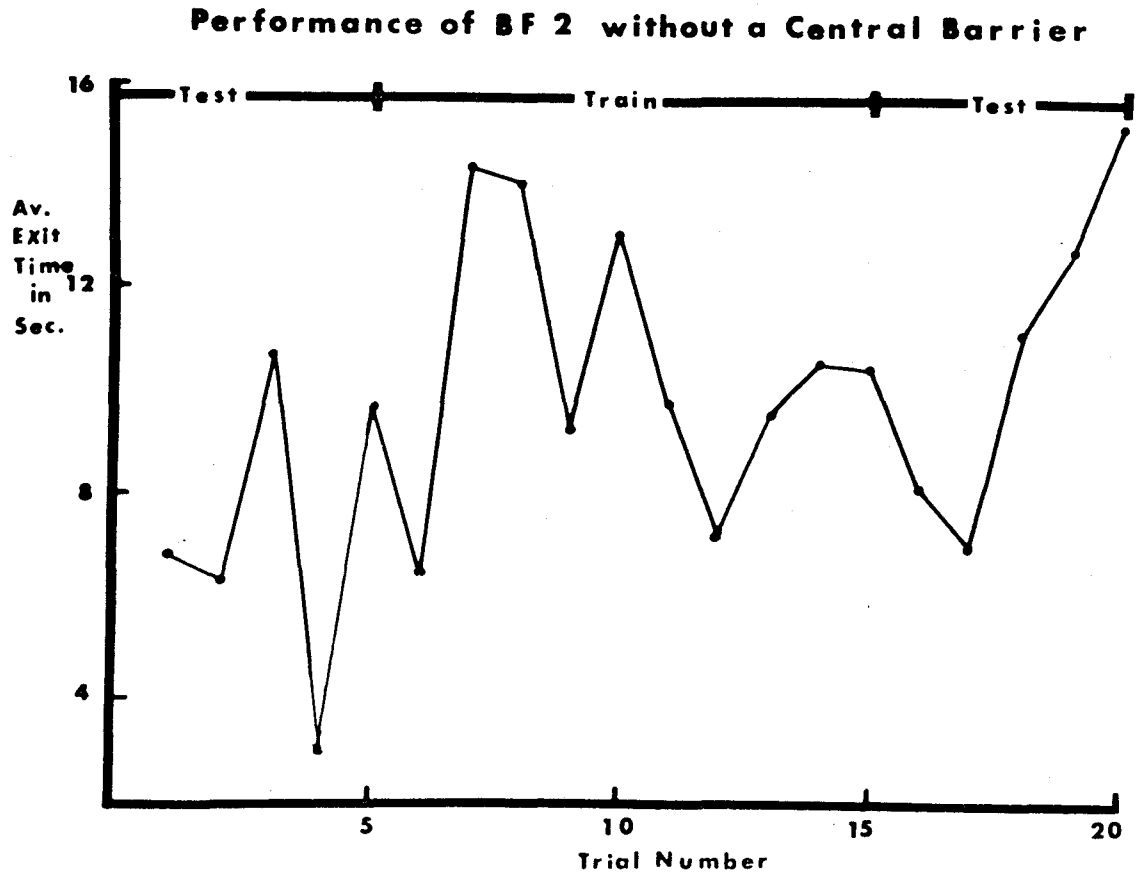

Performance of BF 2 without a Central Barrier
Test
Train
Test
16
12
8
4
Av. Exit Time in Sec.
5
10
15
20
Trial Number

FIGURE 8

LEGEND FOR FIGURE 8.

In this graph the mean exit time for the same trial number of every experimental session is plotted as the ordinate. The corresponding trial number is plotted as the abscissa. Trials one thru five and sixteen thru twenty are "test" trials, while trials six thru fifteen are training trials. Results are for one animal, BF_2, with nine days of training, and one day without training before days eight and nine. No attempt was made to train the animal at the same time each day. No rest periods were included in the protocol. Note, that the first trials following a switch in training procedure had the lower values, as had occurred with BF_1. Note, that additional minima are present which seem unrelated to the protocol; see trials four and twelve. One can also see that the last three trials, eighteen, nineteen and twenty, indicate a progressive deterioration of performance as the average exit time successively increased.

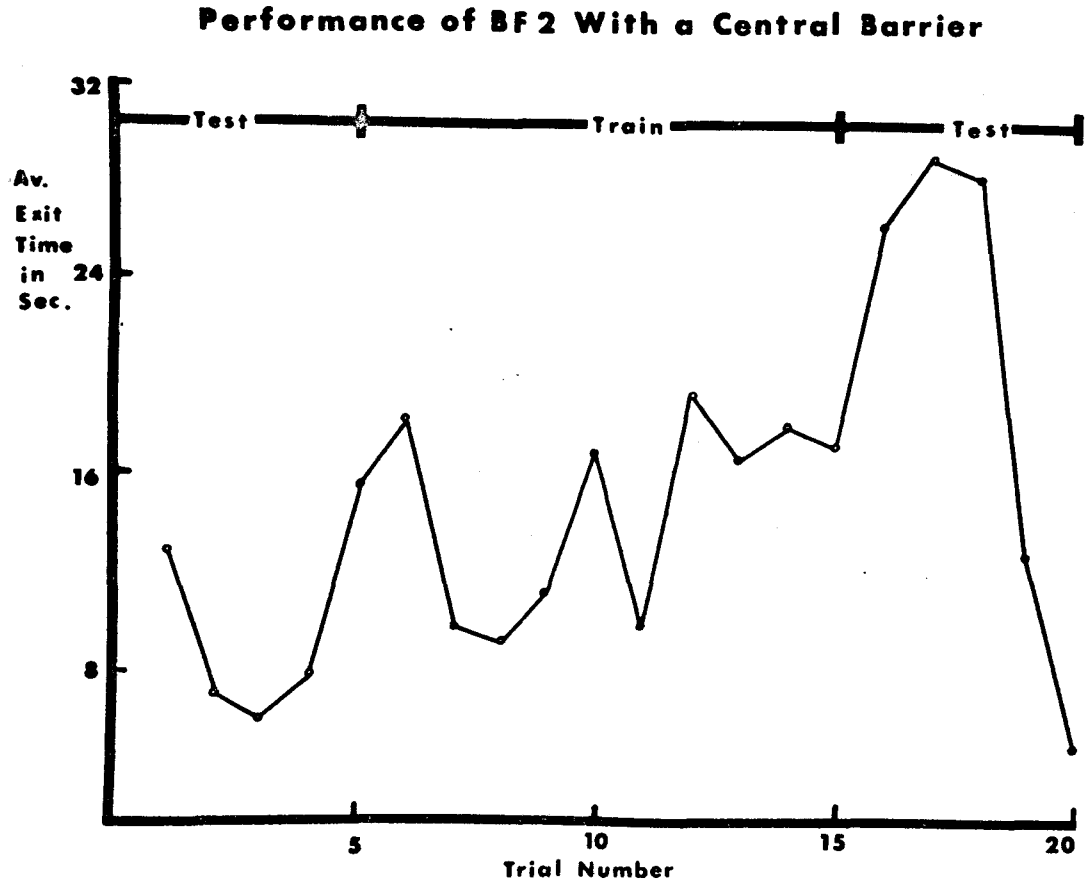

Performance of BF 2 With a Central Barrier
32
Test
Train
Test
Av.
Exit
Time
in 24
Sec.
16
8
5
10
15
20
Trial Number

FIGURE 9

LEGEND FOR FIGURE 9.

The average exit times for the same trial number throughout the experiment is plotted as the ordinate. The appropriate value of the trial number is plotted as the abscissa. The graph depicts the responses of one animal, BF_2, on four days of testing. A test/train/test procedure was used. These values actually indicate the time in seconds that it took the animal to escape the shock. Note, that the last four trials indicate improvement. However trials six and sixteen did not show the decreased exit time values that usually occurred in response to procedural changes at those points. The CS was an overhead red light in the shocking chamber. The UCS voltage was 7 volts. The space between the barriers was opaqued with a sheet of white construction paper.

incorrect since an overall trend towards increased exit times was evident. However, the addition of the barrier may have been responsible for the reversal in the BF's performance on the last four trials; they exhibited progressively lower values and this suggested learning.

The third member in this series, BF_3, (Figure 10) received training trials only. A highly varying performance curve resulted. However, the somewhat lower values for the last seven trials suggested that learning was occurring. A word of caution is indicated here since these aforementioned results consisted of preliminary experimentation on single animals and any observable differences may be due to individual differences rather than procedural differences. On the other hand, the indication that either a three minute rest period or a procedural change yielded better performance is evidenced in the data of BF_1 and BF_2, because the low exit time values always occurred in those trials which followed rest periods. More discussion of this point shall be presented later; see, p. 184.

The consistent fall in exit time values for the last test trials suggested that some adaptive learning might have occurred in BF_1. However it was concluded from the preliminary results that the test trials complicated rather than helped the training. Thus the "test" trials were dropped from the procedure and the training trials were modified to allow for avoidance as well as escape.

The following section of results deals with the avoidance

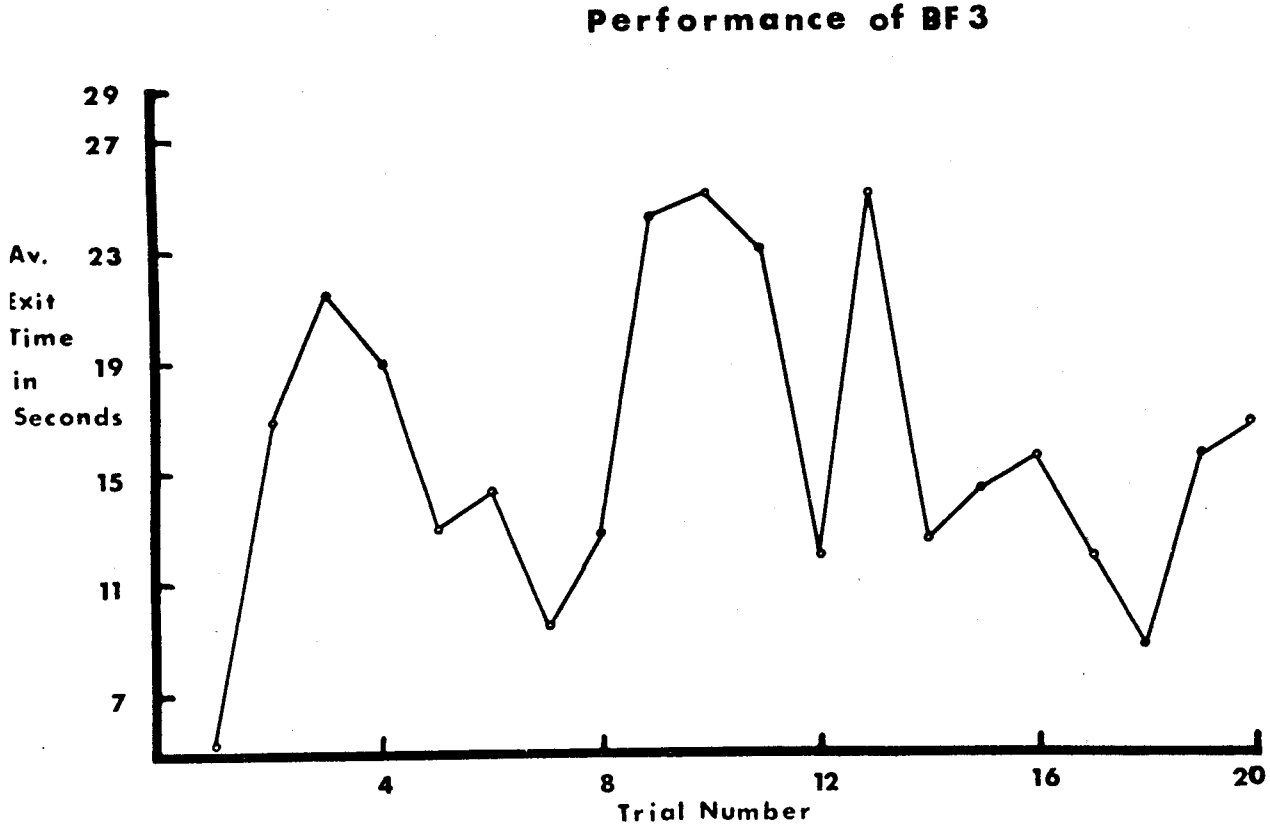

Performance of BF3
29
27
23
19
15
11
7
Av. Exit Time in Seconds
4
8
12
16
20
Trial Number

FIGURE 10

LEGEND FOR FIGURE 10.

The ordinate in this figure represents the average exit time for the same trial number. The associated trial number is plotted as the abscissa. The results are for one animal, BF_3. The experiment continued for nine days with one day off inbetween the eighth and the ninth days. The training procedure consisted of training trials only. The CS was a red overhead light in the shocking chamber which appeared for three seconds prior to shock onset. Following this, the CS and UCS appeared together until the animal crossed into the safe chamber. There were no rest periods interposed between any of the trials and no opaque barrier was interposed between the chambers. The general observed tendency is for exit time values to increase with great variability between trials. The lowest value, fastest escape, occurred on trial one.

conditioning of Rana pipiens. In general two major differences in responses could be readily observed between Rana pipiens and Bufo fowleri. It was plainly obvious that Rana pipiens moved much more quickly than the toads. Secondly, because Rana's skin had a lower electrical resistance they were more acutely sensitive to the electric shock. An effective starting voltage was 3 volts for Rana pipiens compared to 7 volts for Bufo americanus and Bufo fowleri.

The following training procedure was modeled after classical avoidance conditioning procedures, and became the standard procedure in this study; see Figure 4. The intertrial interval was one minute. The CS appeared alone for three seconds in the overhead shocking chamber followed by both the CS and UCS until the animal escaped. The training session was terminated if the animal became incapacitated or unresponsive.

Figure 11 depicts results reminiscent of McGill's (1960) findings. Since the daily mean exit times increased, the level of shock administered had to be increased to maintain performance in Rana pipiens. One of the primary behaviors observed was the persistent hugging of wires by the frogs. Theoretically, this behavior could be an effective way of reducing shock since the making and breaking of contact with the wires, as in jumping around, creates a larger shock (Campbell and Teghtsvonias, 1958). Such behavior is maladaptive since even though the effective level of shock is reduced; the shock still persists and begins

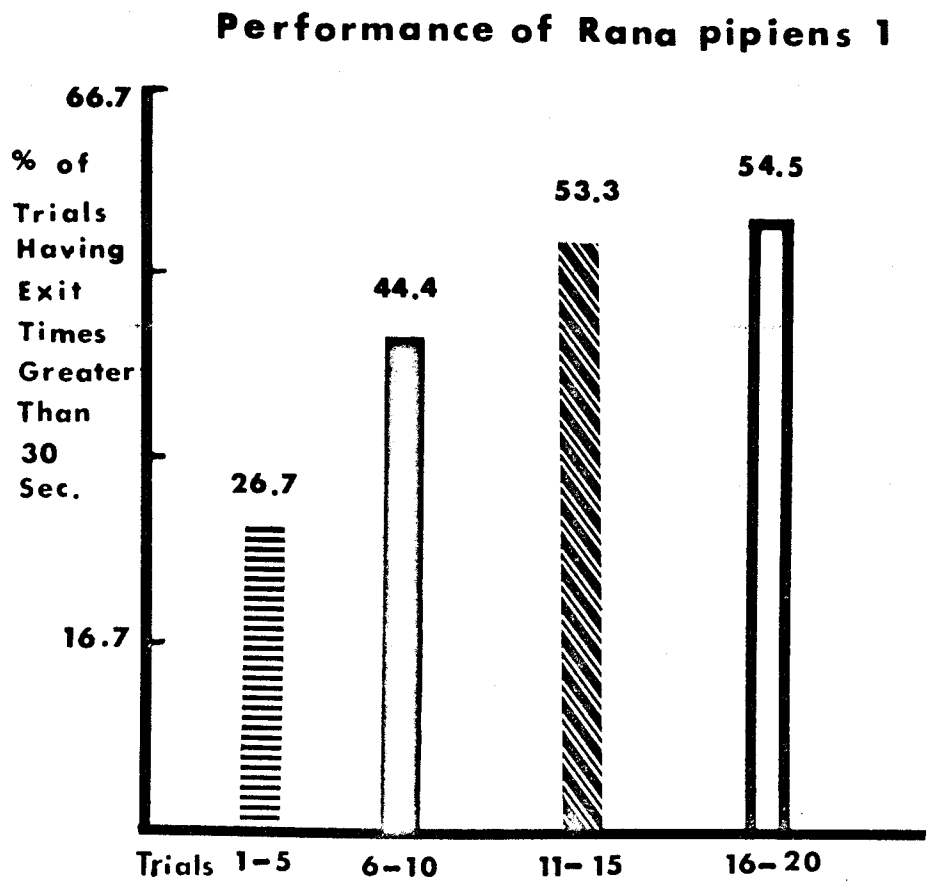


FIGURE 11. The ordinate is the percent of trials with exit times greater than 30 seconds. The abscissa is composed of four blocks of five trials. Each block includes all of the trials experienced during the three days of training. Results are for a single animal, Rana pipiens. These data indicate that with progressive experience the exit times tend to increase. There was no limit for the amount of shock received by the animal except for incapacitation which terminated the session.

to produce gross involuntary muscle twitches in the animal. Once the involuntary muscle twitches occurred, the animal could not move and remained rigid while being shocked. The frog recovered in about five minutes after the shock was terminated. Whenever such an incapacitation occurred the daily session was terminated. Figure 11 indicates how the average exit times increased during daily training.

A second Rana pipiens was started on a modified training procedure where the amount of shock was limited to a maximum of six seconds in any one trial. A total of sixty trials were given on the first day. The voltage was 4 volts derived from the center tap of the transformer. The CS was a white light situated overhead within the shocking chamber; it was on for three seconds prior to the shock. This was followed by the UCS for six seconds unaccompanied by the CS. The frog left the shocking chamber only five times out of the first twenty-one trials and only after the shock had been terminated.

These results gave the impression that the shock was insufficient to cause the frog to escape. Therefore, a slight modification was incorporated. The experimenter alternately switched the shock from 4 to 8 volts about once a second. During trials twenty-one thru thirty-eight the frog exited from the chamber only once after the shock was terminated. The next modification was from trials thirty-nine to forty-two where the duration of shock was increased from six to fifteen seconds, but the shock was

kept at 4 volts. The animal escaped only on trials thirty-nine and forty. Then, beginning with trial forty-three and up to trial fifty-one, a shock duration of fifteen seconds was maintained along with alternating the shock level from 4 to 8 volts. Now the frog crossed seven times out of eight, four of which occurred after the shock terminated. Beginning with trials fifty-two thru sixty, the procedure was modified again maintaining the fifteen second shock duration, but only at 8 volts. This time, the animal crossed six times out of eight, all before the shock terminated, and did not cross the other three times. The three "no crossings" occurred within the last five trials indicating a failure to respond or habituation to the procedure. Fatigue did not seem to be responsible since higher levels of shock temporarily restored performance (McGill, 1960).

The second day of training began with 4 volts of shock lasting for fifteen seconds; otherwise, the procedure was the same. During trials one thru thirteen the frog crossed over into the safe chamber eight times, and only once did the crossing occur after the shock had terminated. The frog did not cross five times, and all of these occurred between trials five thru thirteen. The rest period (intertrial interval) was increased from one minute to one and a half minutes, and the animal did not cross for four consecutive trials (trials fourteen thru seventeen); from trials eighteen thru twenty-three the animal received alternating 4 and 8 volt shocks unlimited in duration

and terminating only after it crossed into the safe chamber. The average exit time for these last six trials was forty-nine seconds past shock onset.

On the third day of training, trials one thru eight had the initial procedure of shock limited to six seconds duration at a level of 4 volts. The frog crossed into the safe chamber only once during the eight trials and again only after the shock had terminated. Then the procedure became shocks of fifteen seconds duration alternating at 4 and 8 volt levels. Now the frog crossed four out of six times; one crossing was after the shock had terminated. The room lights were then turned on hopeful that the animal could see better. This resulted in one crossing and one "no crossing". The procedure was again changed to alternating 4 and 8 volt shocks until the animal crossed; this resulted in an average exit time of fifty-nine seconds for these four trials. Finally, the outside walls were covered with black construction paper to block detracting influences from the room. The room lights still were kept on. The procedure was altered to three seconds of CS overhead in the shocking chamber followed by CS and UCS until the animal crossed. The UCS was again alternating 4 and 8 volt shocks. The mean exit time was 62.5 seconds for the eight trials run this way, and one of these trials had to have the shock terminated manually after 189 seconds.

In summary, then both RP_1 and RP_2 gave responses very clearly reminiscent of the responses observed by McGill; that is

to say, increasing quantities of shock duration and level only temporarily restored performance criteria in these animals. Additional trials led to a deteriorated performance.

A third experiment utilized a Rana pipiens and consisted of the following procedures. The intertrial interval was lengthened from one minute to two and a half minutes. The CS was a white overhead light in the shocking chamber, and the UCS was a shock at 4 volts. The UCS occurred two seconds after the CS began. Both the CS and the UCS continued until the animal left the chamber. Sixty trials were given on each of two days. The data in Figure 12 are represented as the two day average of mean exit time per block of five consecutive trials. One can readily see that from block to block the mean exit time progressively increased. On the second day, the room lights were turned on during the last two blocks of five trials, and it was noted that a significant decrease in exit times occurred. This decrease was statistically significant with $p<.05$ via the unpaired, one tailed, Student t-test. These results reinforced the findings of McGill; however, they implied that the ambient level of illumination could be of critical importance. The importance of the level of ambient illumination in the conditioning of anura is explored further in another section of this thesis.

Similarly, a group of Rana pipiens from Vermont were examined for their conditioning abilities as well. RP_6V_1 underwent a single daily session of twenty-six trials. The procedure

was an intertrial interval of one minute and the CS appeared overhead in the shocking chamber for three seconds; this was followed by both the CS and UCS at 4 volts until the animal escaped. This animal, RP_6V_1, also showed a performance decrement with progressive experience.

The second member of the Vermont variety was given the following familiar procedures from trials one thru sixty-seven: the intertrial interval was one minute, the overhead CS appeared in the shocking chamber for three seconds, then both the CS and UCS at 4 volts continued for an additional six seconds (Procedure A). From trial sixty-seven thru trial ninety, the UCS was alternated between the 4 and 8 volt levels for a period of fifteen seconds (Procedure B). The outside walls were covered with black paper and the center barrier was covered with yellow. The data are represented in Figure 13 as the total time that the animal received shock during each block of five trials. It can be seen in Figure 13 that the total amount of shock per block in the first procedure stayed relatively close to the maximum of six seconds; concurrently, the animal left the chamber prior to conclusion of the shock an average of only 1.36 times per block. On the other hand, in Procedure B the amount of shock received was greater, but not quite near the maximum of fifteen seconds for this procedure. The shock remained an effective stimulus for the next twenty-five trials. In Procedure B the animal left the chamber, before the shock terminated, an average of 4.2 times

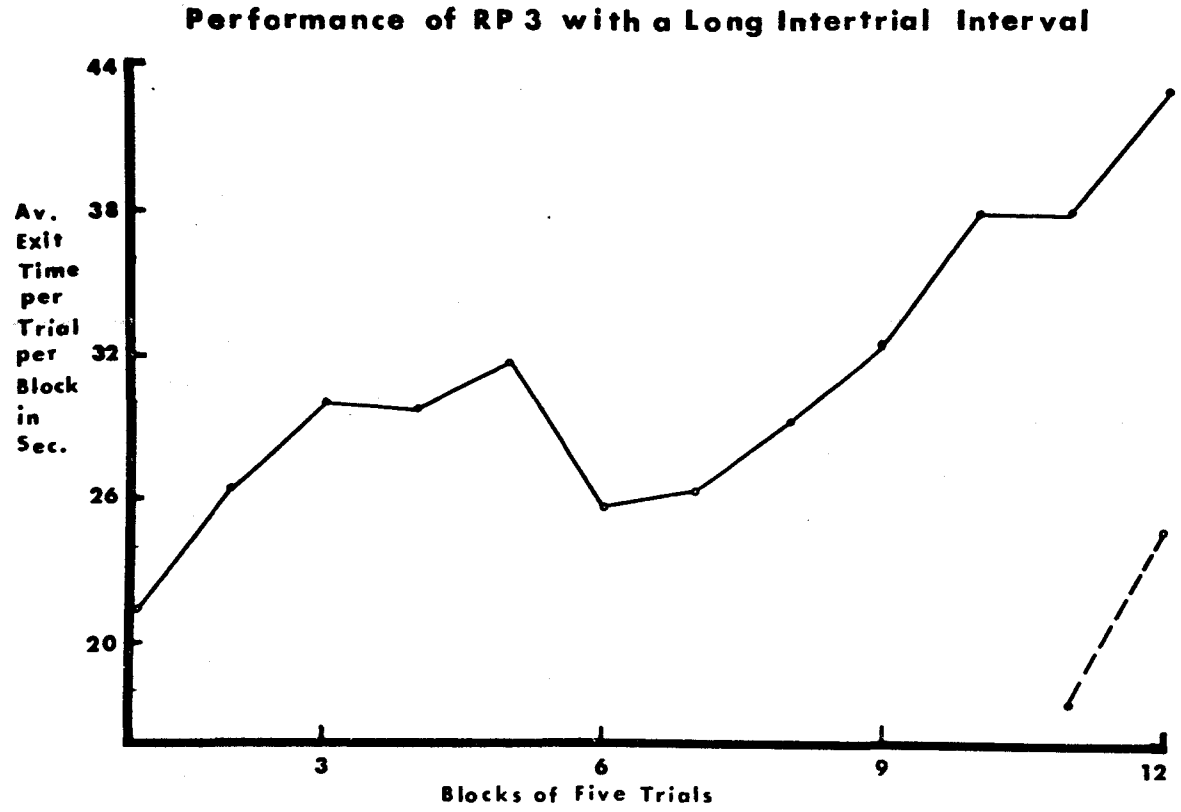


FIGURE 12. This figure has the average exit time per block of five trials as the ordinate with the block number as the abscissa. The total number of trials given each day was sixty. It can be seen that a pronounced tendency exists for the exit time values to increase with continued experience. The graph depicts the experience with one animal. The room lights were turned on during blocks eleven and twelve for the second day. This produced a significant difference in response with $p<0.05$ by the one tailed student t-test (dashed line, o---o, indicates the lights on situation).

per block. Again this reinforced the previous data that additional shock can motivate the frog to respond. Thus, performance can be maintained in Rana if the intertrial interval is long enough, or if the shock is limited.

The last Rana pipiens studied, RPV_3, had five minute rest periods interspaced between each block of five trials. The basic design was the same as Procedure A of the previous frog discussed. In comparing the results of the first twelve blocks of training for RPV_2 and of RPV_3, there seems to be no major influence of long rest periods; see Figure 14.

The following two experiments investigated the value of the rest periods. Bufo fowleri 5 was trained for a day with the following procedure: the intertrial interval was one minute, and the CS had a duration of two seconds and was a white light overhead in the shocking chamber. This was followed by both the UCS and CS until escape. A rest period, twenty minutes duration, was interspaced between each block of ten trials. A total of four blocks of ten trials were undertaken (forty total trials). Figure 15 depicts the results. It can readily be seen that the lowest exit time values always occurred after a rest period. Furthermore, the trend towards longer exit times in the later trials also continued as it had in the previous experiments.

The second experiment with a member of the species Bufo americanus 2, BA_2, followed the same basic pattern as the previous experiment with BF_5. Except for the two different

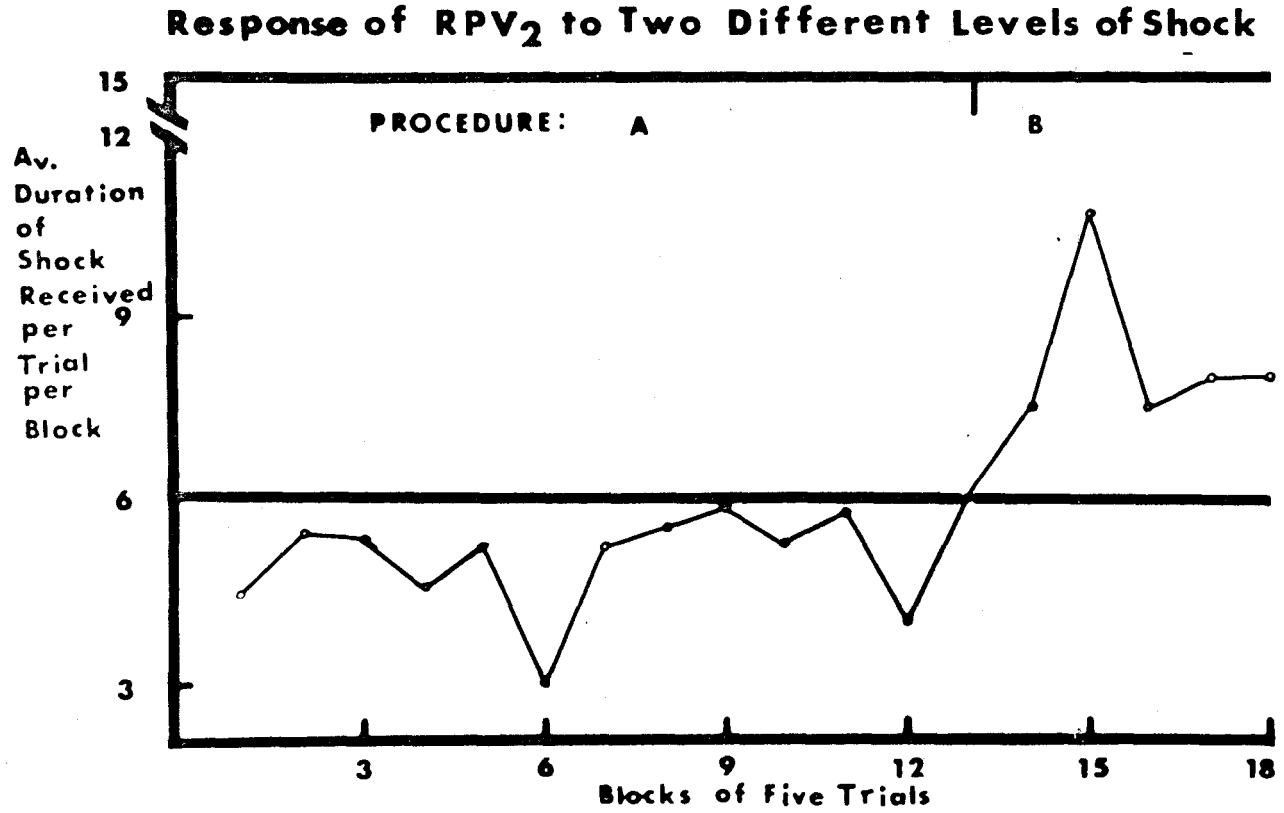

Response of RPV2 to Two Different Levels of Shock
PROCEDURE: A
B
15
12
9
6
3
Av. Duration of Shock Received per Trial per Block
3
6
9
12
15
18
Blocks of Five Trials

FIGURE 13

LEGEND FOR FIGURE 13.

The ordinate is the average amount of shock received per trial calculated by averaging five consecutive trials. Six seconds was the maximum time that shock could be received in Procedure A. Fifteen seconds was the maximum amount of time that shock could be received in Procedure B. The Procedures A and B are described in the text. Results are for one animal and one day's training.

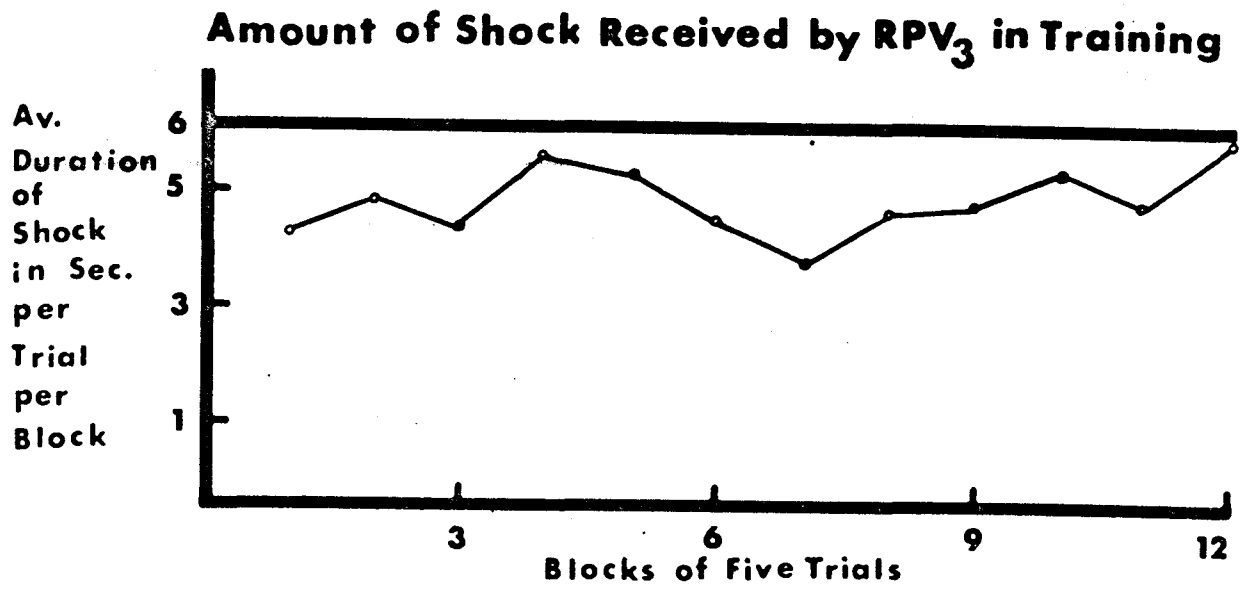


FIGURE 14. The ordinate is the total amount of shock received per block of five trials. The abscissa is composed of the consecutive blocks of five trials. Results are depicted for one animal, a Rana pipiens Vermont variety. Rest periods of five minutes duration were given in between each block of five trials. The shock level was 3 volts and was limited to a maximum of six seconds duration per trial. Note, that the rest periods did not improve the animal's performance and did not produce consistent responses. Learning would be considered to be a decrease in the mean exit times per consecutive blocks.

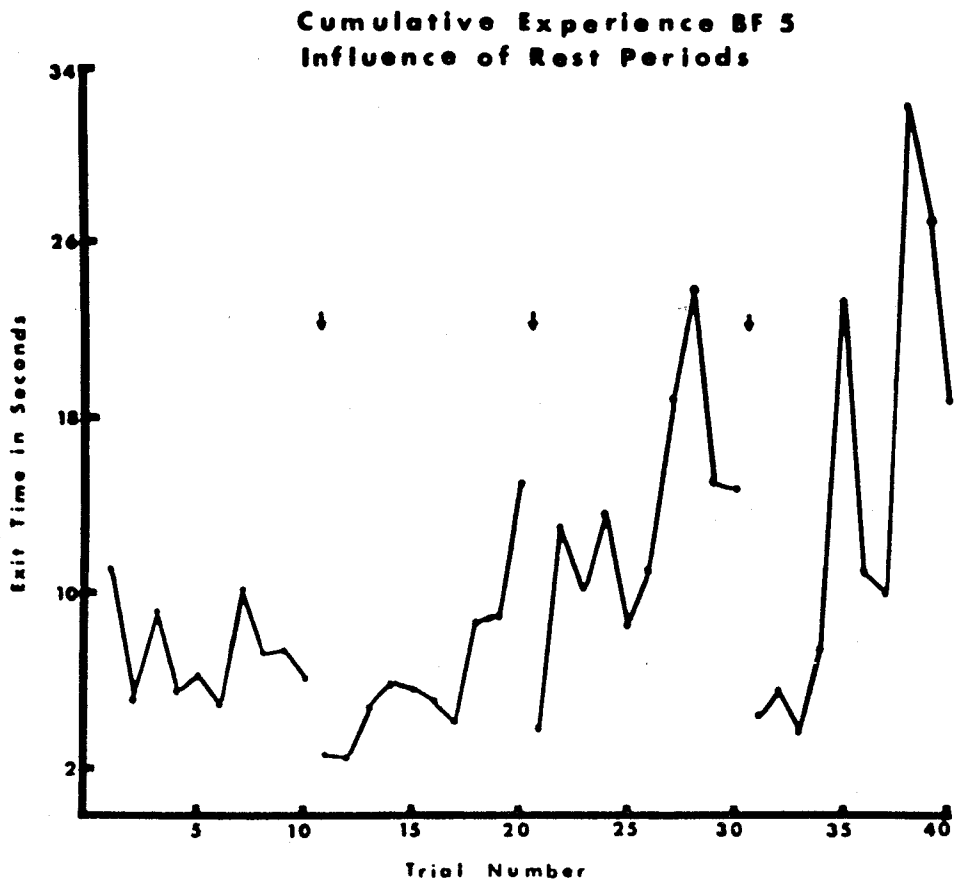


FIGURE 15. The exit time for each trial is the ordinate. The abscissa is composed of the consecutive trial number, and is a measure progressive experience with the apparatus. The arrows indicate twenty minute rest periods. Note, the lowest exit time values occur immediately after the rest periods. The general tendency towards longer exit times with progressive experience is also obvious.

intertrial intervals one of twenty seconds and the other of one minute, the following parameters were the same. The CS duration was two seconds and was immediately followed by both the CS and the UCS simultaneously until escape. The shorter intertrial interval was used, at first, because the animal was very active in the first trials, and with the one minute interval the animal tended to cross into the safe chamber before the CS began. One can see, in Figure 16, that the trials immediately following the rest periods had the lowest exit time values, and that this was independent of the duration of the intertrial interval.

Experiment 7 was carried out with a single animal giving it many trials as possible for three days. The results were tabulated to see if a significant change in exit times had occurred. These data are plotted as groups of five trials. Figure 17 indicates that at no time did the figures dip below the initial exit time value. Therefore, one could not say that the addition of more training trials per day could have yielded better performance after three days of training since the exit time values had a high variability.

Subsequently, it was realized that the spontaneous crossings were avoidance responses and not mistrials. Therefore, spontaneous crossing activity was also recorded and analysed for changes during training. Two animals were used in this experiment: BF_6 who had the CS appear in the shocking chamber, and BF_{11} who had the CS appear in the safe chamber. The results are depicted in

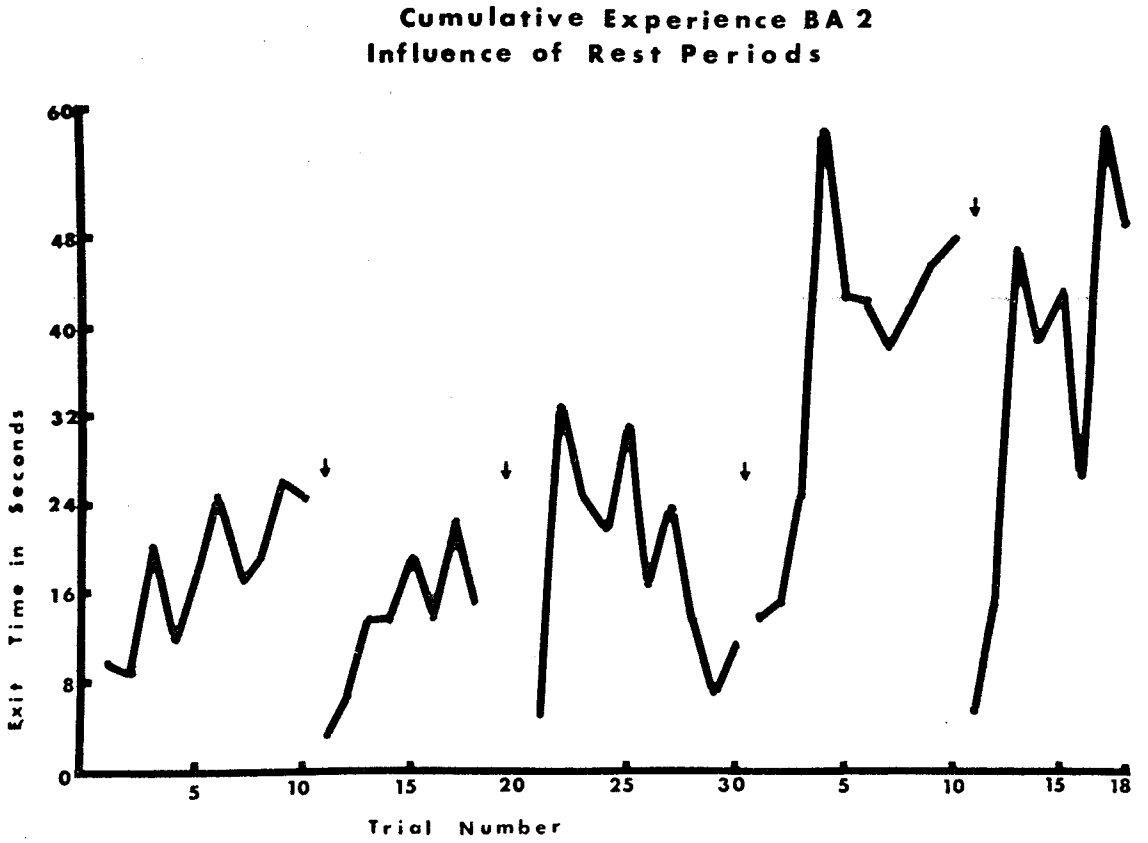


FIGURE 16. The ordinate in this figure depicts the exit time for each trial. The abscissa is the consecutive trial number. The arrows indicate twenty minute rest periods. The intertrial interval during trials one thru thirty was twenty seconds. The intertrial interval was one minute for the last eighteen trials.

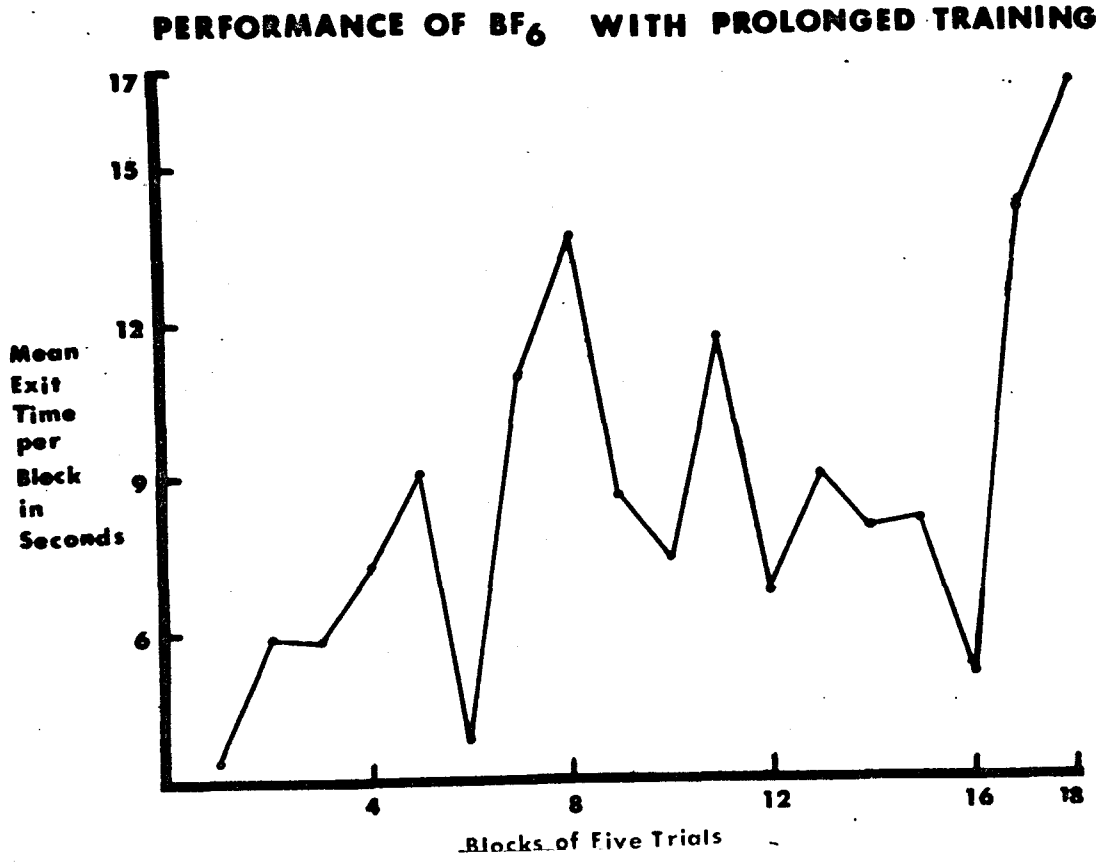

PERFORMANCE OF BF6 WITH PROLONGED TRAINING
17
15
12
9
6
Mean Exit Time per Block in Seconds
4
8
12
16
18
Blocks of Five Trials

FIGURE 17

LEGEND FOR FIGURE 17.

One animal, BF_6, was given ninety trials per day on a "training only" schedule. The intertrial interval was one minute. The CS duration was three seconds and both the CS and UCS were given either until the animal escaped or until a maximum duration of sixty seconds. Training continued for three consecutive days. The average exit times per trial are plotted as the ordinate. This value was calculated from the values obtained from a block of five consecutive trials. The analagous blocks from the daily sessions were pooled together for the calculation. The abscissa is the appropriate block of trials corresponding to the plotted exit time value. One can see that no simple relationship exits between these two measurements. In fact at no time did the exit time values fall below the initial value for trials one thru five.

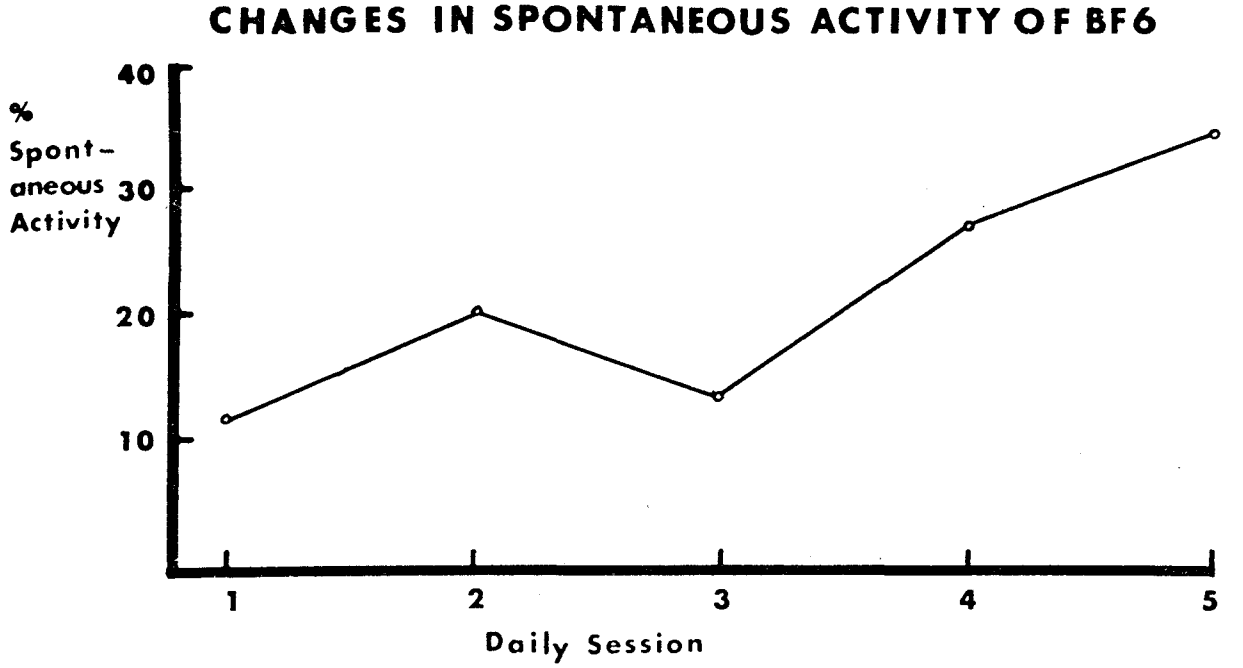


FIGURE 18. The ordinate is the percent of all trials per daily session where spontaneous crossings were observed. The abscissa is the day of training. Data are for one animal, BF_6. The CS appeared in the shocking chamber three seconds prior to shock. CS was white in color. The outside of the chamber was covered with black construction paper. The outside of the far chamber wall was covered with blue construction paper. The intertrial interval was one minute. No wires remained at the base of the center section. Ten trials were given per day for five days. Spontaneous activity is synonymous with spontaneous crossings.

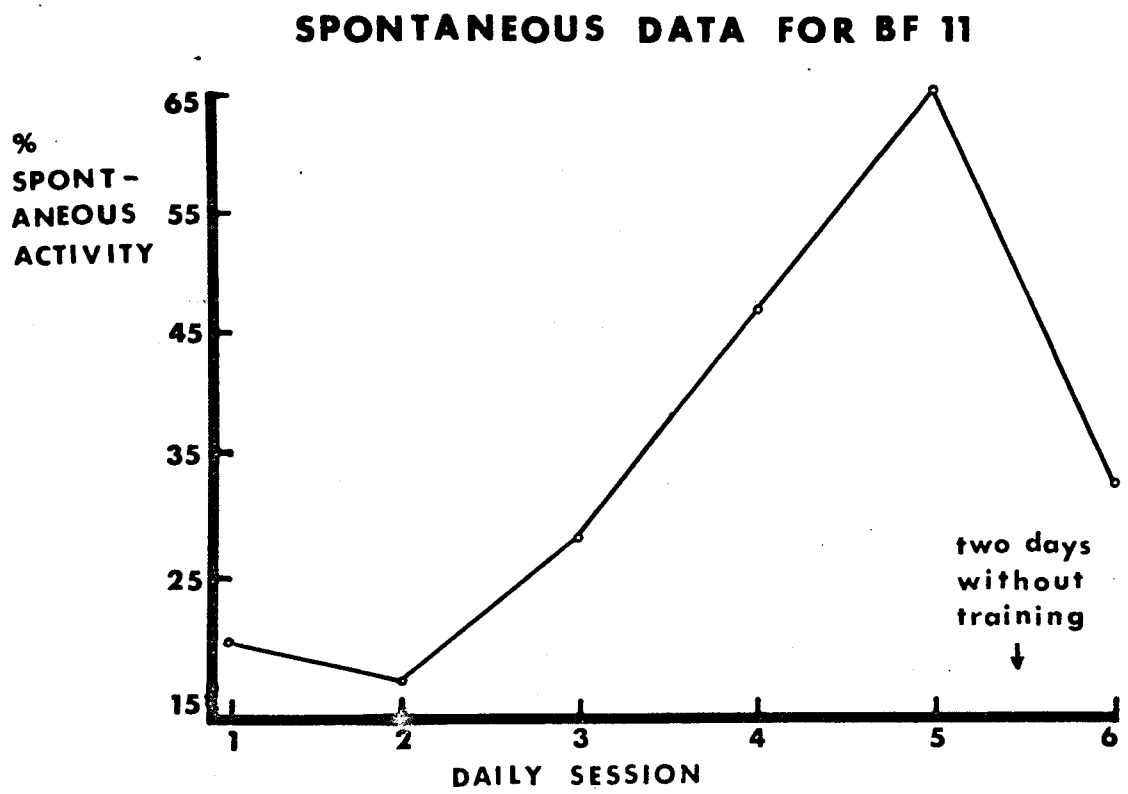


FIGURE 19. The ordinate is the percent of all daily trials where spontaneous crossing was observed. The abscissa is the day of training. Data are for one animal BF_{11}. The CS appeared in the safe chamber for three seconds prior to shock. The CS was white in color. The outside of the chamber was covered with black construction paper. The far wall of the chamber was covered with blue construction paper on the outside. The intertrial interval was one minute. No wires remained at the base of the center section. Ten trials were given per day for six days. Two days without training occurred between training days five and six. Spontaneous activity is used synonymously with spontaneous crossing.

Figures 18 and 19. Both animals showed an increase in spontaneous crossing by the end of the experiment, which had lasted five days. This observation suggested that a non-classical form of learning was occurring. Therefore, all spontaneous crossing which occurred in the subsequent experimentation was recorded as data.

Other evidence that spontaneous crossing was an important behavior to quantitate came out in experiment six. Five animals were included: BA_6, BA_7, RPV_4, BF_{12} and BM_5, all were trained with the same procedure as described in the Methods Section under experiment 6. BA_6, RPV_4 and BM_5 were trained with the room lights on. BA_7 and BF_{12} were trained with the room lights off. Figure 20 illustrates the percent of total daily trials which were spontaneous crossings. Rana pipiens did not display any spontaneous crossings and is not included in the figure. The other two who were trained with the room lights on, namely BA_6 and BM_5, had higher levels of spontaneous crossing occurring towards the end of training, days three, four and five, than in the beginning of training, days one and two. BA_7 and BF_{12} showed variability in their daily responses. It was implied from these results that having the room lights on aided the acquisition of an adaptive behavioral pattern which was arbitrarily called either spontaneous activity or spontaneous crossing. Similarly in Figures 21 and 22, one can see that the total exit time decreased from theoretical values for BA_6 and

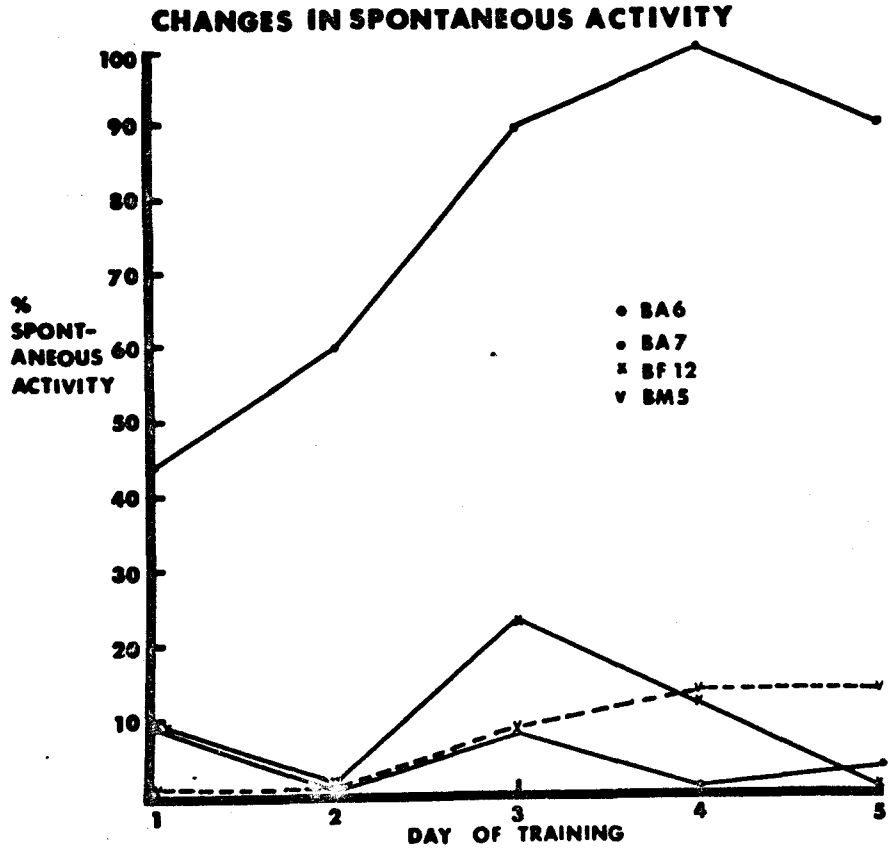

CHANGES IN SPONTANEOUS ACTIVITY
% SPONT-ANEOUS ACTIVITY
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
DAY OF TRAINING
• BA6
• BA7
x BF12
v BM5

FIGURE 20

LEGEND FOR FIGURE 20.

The ordinate is the percent of spontaneous crossings observed per daily session. The abscissa is the day of training. Three species are represented BA_6 (•) and BA_7 (°), BF_{12} (x) and BM_5 (✓). A fourth species Rana pipiens showed no spontaneous activity and is excluded from the figure. BA_6, BM_5 and Rana pipiens were all trained with the room lights on, while BA_7 and BF_{12} were trained with the room lights off. An increase in the frequency of occurrence of spontaneous crossings occurred for BA_6 and BM_5. The CS which was white in color appeared overhead in the safe chamber. The CS duration was three seconds. The inside wires were exposed. The outside, side walls and base were covered with black construction paper. The outside far wall was covered with blue construction paper. The intertrial interval was one minute. The shocking voltage was 7 volts. From ten to twenty trials were given per daily session. Training continued for five consecutive days.

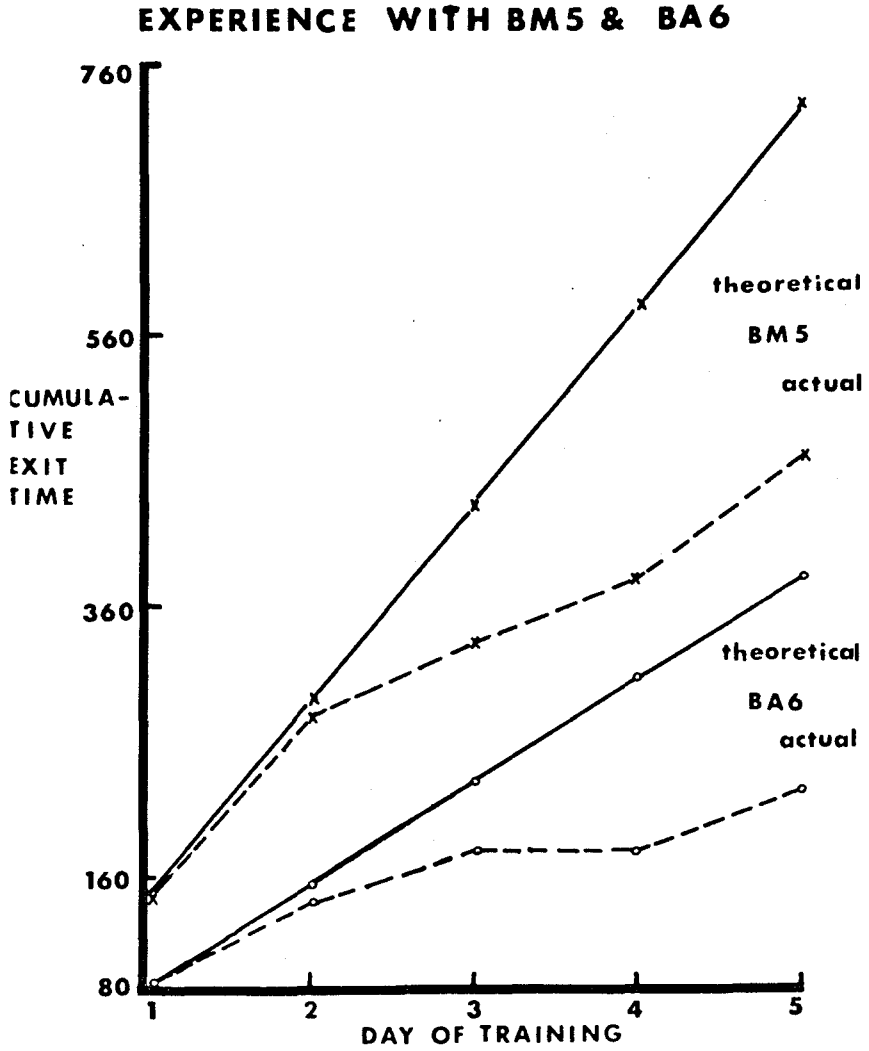

EXPERIENCE WITH BM5 & BA6
760
560
360
160
80
CUMULA-
TIVE
EXIT
TIME
theoretical
BM5
actual
theoretical
BA6
actual
1
2
3
4
5
DAY OF TRAINING

FIGURE 21

LEGEND FOR FIGURE 21.

The ordinate is the sum total in seconds of all exit times which occurred for the first ten trials of a daily session. The average for the first ten trials excluding mistrials was calculated, multiplied by ten, and added to the previous days total. The theoretical curve uses the first day as a control level. One, then, assumes that the first ten trials on day one are representative of the actions of the animal in the experimental situation, and should be repeated every day that the animal encounters the apparatus. Therefore, subsequent values of the theoretical curve, that is for days of training two, three, etc., are actually the first day's response added to itself.

The actual curves represent the total exit times calculated from the average value of the first ten trials of each daily session; this was added to the previous day's value. If the actual curve moves to the left of the theoretical curve the animal is showing longer exit times. If the actual curve moves to the right of the theoretical curve the animal is responding more rapidly to the shock. This movement of the curve to the right suggests a trend towards adaptive learning by the animal. Theoretical curve for BM_5 (x——x); actual curve for BM_5 (x---x); theoretical curve for BA_6 (°——°); actual curve for BA_6 (°---°).

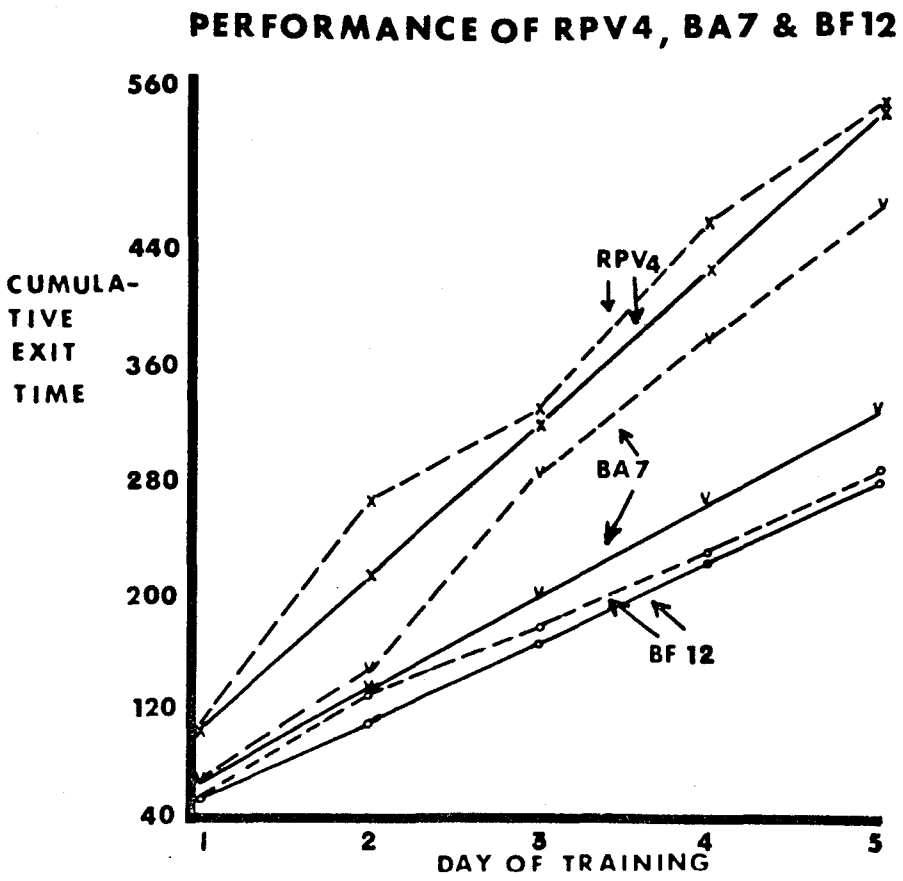


FIGURE 22. The basic description is the same as the legend for Figure 19. Theoretical curve for RPV_4 (x——x); actual curve for RPV_4 (x---x); theoretical curve for BA_7 (v——v); actual curve for BA_7 (v---v). Theoretical curve for BF_{12} (°——°); actual curve for BF_{12} (°---°). All animals represented in this figure showed a tendency towards longer exit times over the course of training.

BM_5 as compared to BA_7, BF_{12}, and RPV_4 where it increased.

The methods of experiment 8 were discussed in the Methods Section of this thesis. Taking the lead that spontaneous activity was the best measure of learning in these animals, experiment 8 was designed to study the difference between two conditioned stimuli. One conditioned stimulus appeared in the overhead plane of the "safe" chamber, the "overhead CS" group, while the other CS was a flashing light in the safe chamber and situated at approximately eye level, the "flashing CS" group. One group was trained without any CS, the "no CS" group. Five animals were included in each group. Figure 23 depicts the average exit time per each trial. The "overhead CS" group which is represented by the solid line showed an increased variability of response over the course of the training session. The "no CS" group and the "flashing CS" group showed an increased variability in response, and a slight general tendency for exit times to increase with continued training. What is most important though, is that the values never approached the three second mark. Any value less than or equal to three seconds would have been classified as an avoidance response.

Another analysis to determine whether the groups varied significantly from each other at the start of the experiment is depicted in Table 1. It can be seen from the two tailed unpaired student t test that the difference in naive exit times for trial one day one are not statistically significant.

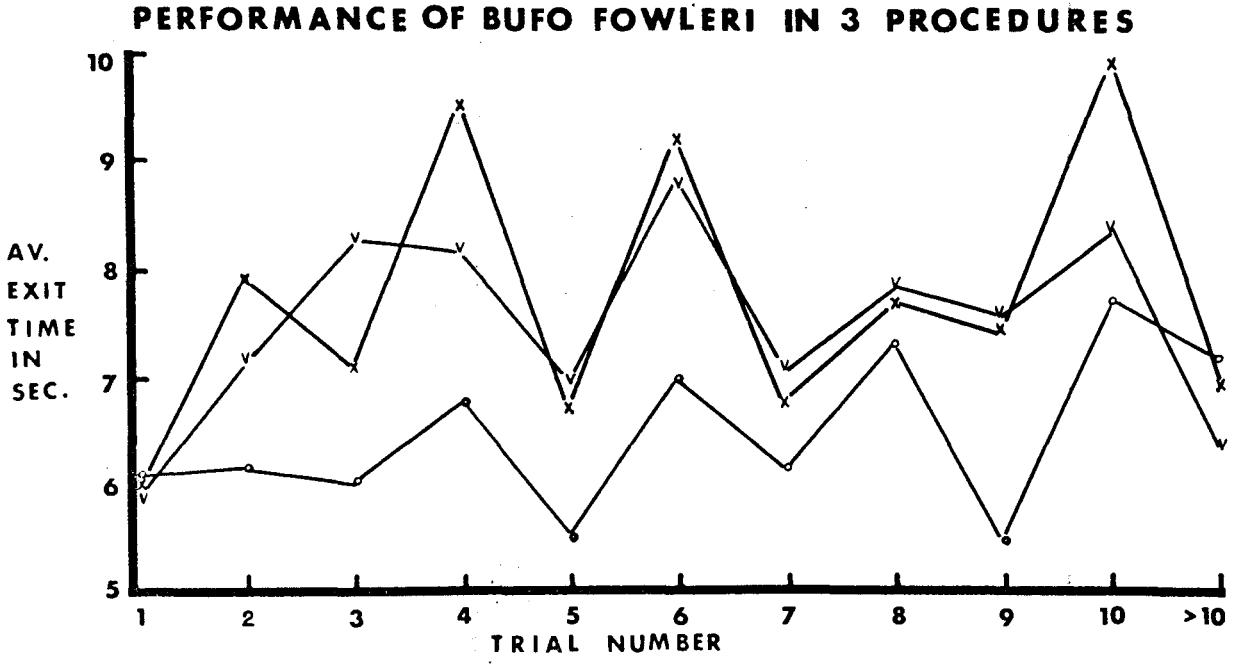

PERFORMANCE OF BUFO FOWLERI IN 3 PROCEDURES
AV. EXIT TIME IN SEC.
10
9
8
7
6
5
1
2
3
4
5
6
7
8
9
10
>10
TRIAL NUMBER

FIGURE 23

LEGEND FOR FIGURE 23.

This figure has as its ordinate the average exit time per trial number per group. The abscissa is the corresponding trial number. The first ten trials are plotted while the rest are grouped together and represented as greater than ten. Group I, GI and (°——°), had a CS appear overhead in the safe chamber. Group II, GII and (v——v), had no CS cue. Group III, GIII and (x——x), had a CS at eye level in the safe chamber and flashing at a rate of twice a second. If any average value or individual value was less than three seconds it was considered to be avoidance. The intertrial interval was one minute. The CS was a white light and appeared for three seconds. The CS was paired with UCS following either the CS or the no CS time period, three seconds, and continued until the animal escaped or the elapsed time was one minute. Each point represents an average of twenty-five values, five animals and five days of training. All groups were trained and tested without the room lights on. The overhead CS group, GI, appears to have had the fastest response. See Table 1 p. 121 and Table 2 p. 122 for statistical test.

TABLE 1

COMPARISON OF GROUPS AT START OF EXPERIMENT *

COMPARISON	t VALUE	TOTAL NO. OF VALUES	DEGREES OF FREEDOM	P VALUE	SIGNI-FICANCE
Group I vs Group II	1.0332	10	8	$p > .2$	N.S.
Group I vs Group III	1.1730	10	8	$p > .2$	N.S.
Group II vs Group III	1.4521	10	8	$p > .1$	N.S.

* Unpaired student t-test, two tailed

LEGEND FOR TABLE 1

The naive exit time value is the value recorded on trial one of the first experimental training session. These naive values were pooled for each group (five values per group) and were compared to the other groups. The unpaired two tailed student t-test was used to test the null hypothesis that there was no significant difference between the populations of naive values recorded for each group. A "p" value ≤ 0.05 was the criteria upon which the judgement of significance was made. For all groups the null hypothesis is accepted, and no difference between the groups could be detected by examination of their naive exit times. Group I (CS) had a mean exit time for trial 1 of 4.51 seconds. Group II (No CS) had a mean exit time for trial 1 of 5.58 seconds. Group III (Flashing CS) had a mean exit time for trial 1 of 4.10 seconds. This table refers to Figure 23.

TABLE 2

COMPARISON OF GROUPS AT END OF EXPERIMENT
WITH RESPECT TO TOTAL MEAN EXIT TIME **

COMPARISON	GROUP	NO. OF ANIMALS	NO. OF VALUES	MEAN EXIT TIME	STANDARD DEVIATION OF MEAN	DIFFERENCE*	t	d.f.	p	SIGNIFICANCE
A	I	5	243	6.82	1.8904	0.71	2.7344	429	<.005	Sig.
	II	5	248	7.53	3.5891					
B	I	5	243	6.82	1.8904	1.16	3.5704	487	<.001	Sig.
	III	5	246	7.78	3.7466					
C	II	5	248	7.53	3.5891	0.25	0.7574	492	< 0.3	N.S.
	III	5	246	7.78	3.7466					

** Unpaired one tailed student t-test
* In seconds

LEGEND FOR TABLE 2

The most reliable indicator of exit times is the mean exit time for all good trials during the experiment. If any difference exists between the groups it can be detected by examining these values. For Compairson A, the Null hypothesis was that the mean exit time for Group I was not less than, but the same as the mean exit time for Group II. A "p" value $<.05$ is the criteria by which the null hypothesis is rejected. The "p" value is $<.005$. Therefore, the null hypothesis is rejected, and we have evidence that Group I had a lower mean exit time than Group II with only 1:200 chance that the difference was due to chance alone. For Comparison B, the Null hypothesis was that the mean exit time for Group I was not less than that of Group III. The "p" value is $<.001$, therefore, the null hypothesis is rejected. For Comparison C, the Null hypothesis was that the mean exit time for Group II was not less than that of Group III. The "p" value is $<.3$, therefore, the null hypothesis is accepted since we have more than a 5% chance error that this difference could be due to chance alone. Thus, Group I escaped faster than either Group II or Group III. However, no significant difference in escape times could be detected between Groups II or III. This table is with reference to Figure 23.

Figure 23 suggests that the "overhead CS" group was faster than the other groups. The averaged exit times for all trials of the "overhead CS" group are lower than those for either "no CS" or the "flashing CS" group; see Table 2. There was a statistically significant difference between the lower values for the "overhead CS" group when compared to the group without any CS, $t = 2.7344$, $p < .005$, 429 d.f., using the unpaired one tailed Student t-test. Furthermore, the values for the"overhead CS" group when compared to the "flashing CS" group, also showed the mean for the "overhead CS" group to be lower. This difference also was statistically significant $p < 0.001$, 487 d.f., $t = 3.5704$, from the unpaired one tailed Student t-test. Also, by the same test it was found that the observed difference between the "no CS" group and the "flashing CS" group was not statistically significant, $t = 0.7574$, 492 d.f., $p < 0.3$. See p.190 for the interpretation of data and suggested biological significance.

The data listed in Table 3 indicated that the mean exit time for the last five trials, trials six through ten, was always larger, than the value for trials one through five. Also by the one tailed unpaired Student t-test, it was found that the larger mean exit time for trials six through ten of the group with the "flashing CS" was significantly different from the lower mean exit time for trials one thru five. The group with "no CS" had an almost significant difference between the

mean exit time values for trials one thru five and six thru ten with $p < .08$ in the one tailed t-test.

With regard to spontaneous crossings (Figure 24) only the "no CS" group and the "flashing CS" group showed an increased adaptive performance with continued experience. The "overhead CS" group shows an unexpected result of a decreased level of spontaneous crossings. Figure 25 and Table 4 depict the total difference in frequency of occurrence of spontaneous crossings for all groups. The value of $x^2 = 8.2078$ with $p < .05$ indicated that there is a good chance for a real difference between the three groups. For some unexplainable reason, however, the "no CS" group displayed a greater degree of spontaneous crossings than either of the other two groups. One might venture to say that the CS had a detrimental effect in those groups. The Binomial t-test was used to compare within a group the frequencies of occurrence for the first five trials and the second five trials. There was a statistically significant difference between trials one thru five and trials six thru ten in the "overhead CS" group (one tail $t = 1.66$ with $p < .05$), but no statistically significant difference in either of the other two groups. These results are depicted in Figure 26.

Experiment 9 basically consisted of subjects from two species, Bufo fowleri and Bufo americanus. The major change in the apparatus was to line the inside walls with construction paper as described in the Methods Section; see p. 73.

TABLE 3

COMPARISON FOR EACH GROUP OF EXIT TIMES IN LAST HALF OF TRAINING SESSION TO THOSE OF THE FIRST HALF **

GROUP	TRIALS	NO. OF ANIMALS	NUMBER OF OBSERVATIONS	MEAN EXIT TIME	STANDARD DEVIATION OF MEAN	DIFFERENCE	t	d.f.	p	SIG
I	1-5	5	109	6.71	1.0656	0.09	0.3797	218	< 0.4	N.S.
	6-10	5	111	6.80	2.2403					
II	1-5	5	118	7.29	3.6743	0.68	1.4116	228	<.08	N.S.
	6-10	5	112	7.97	3.6291					
III	1-5	5	111	7.44	3.7165	0.87	1.7062	220	<.05	Sig.
	6-10	5	111	8.31	3.8805					

** Unpaired one tailed student t-test
* In seconds

LEGEND FOR TABLE 3

The null hypothesis for each group is that the mean exit times for trials six through ten are not greater than for trials one through five. The unpaired one tailed student t-test was used. A "p" value $< .05$ indicates a significant difference. Only in Group III was the null hypothesis rejected. Therefore, there is evidence that the exit times for trials six through ten are greater than trials one through five only in Group III, the flashing CS group. This figure refers to Figure 23.

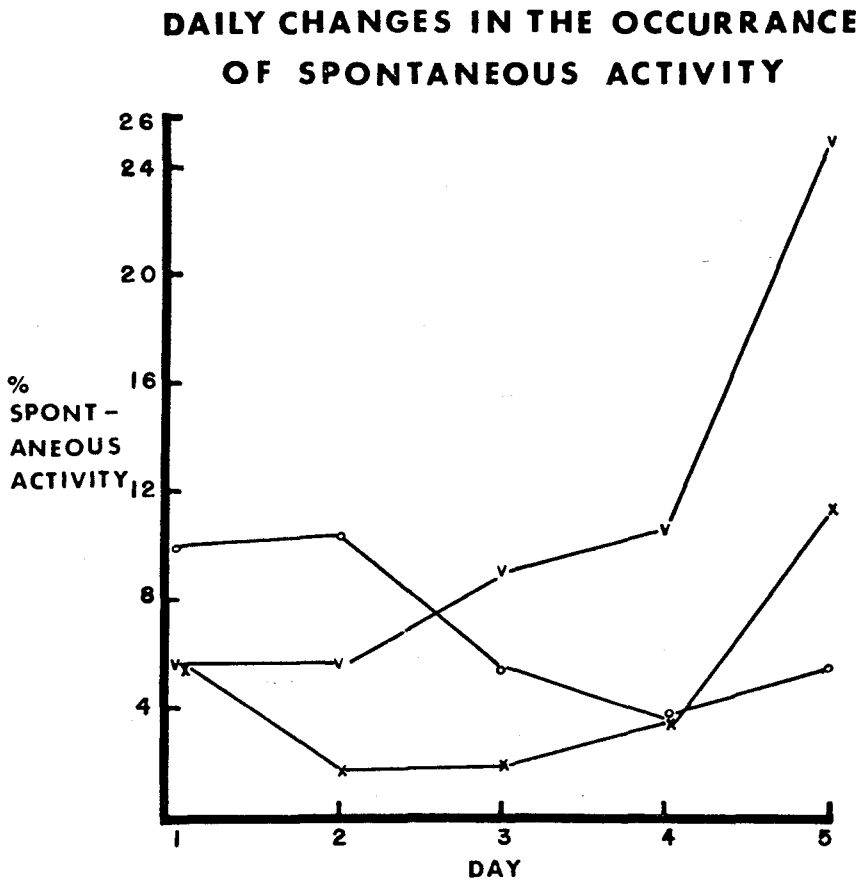


FIGURE 24. The ordinate is the percent of spontaneous activity observed per daily session. The abscissa is the day of training. Each group is represented on the graph. Group I with CS overhead in safe chamber is depicted as (°——°). Group II with no CS cue is depicted as (v——v). Group III with CS flashing at two per second is depicted as (x——x). It appears that improved performance, avoidance of the shock via a spontaneous crossing during the intertrial interval, occurred only in Groups II and III.

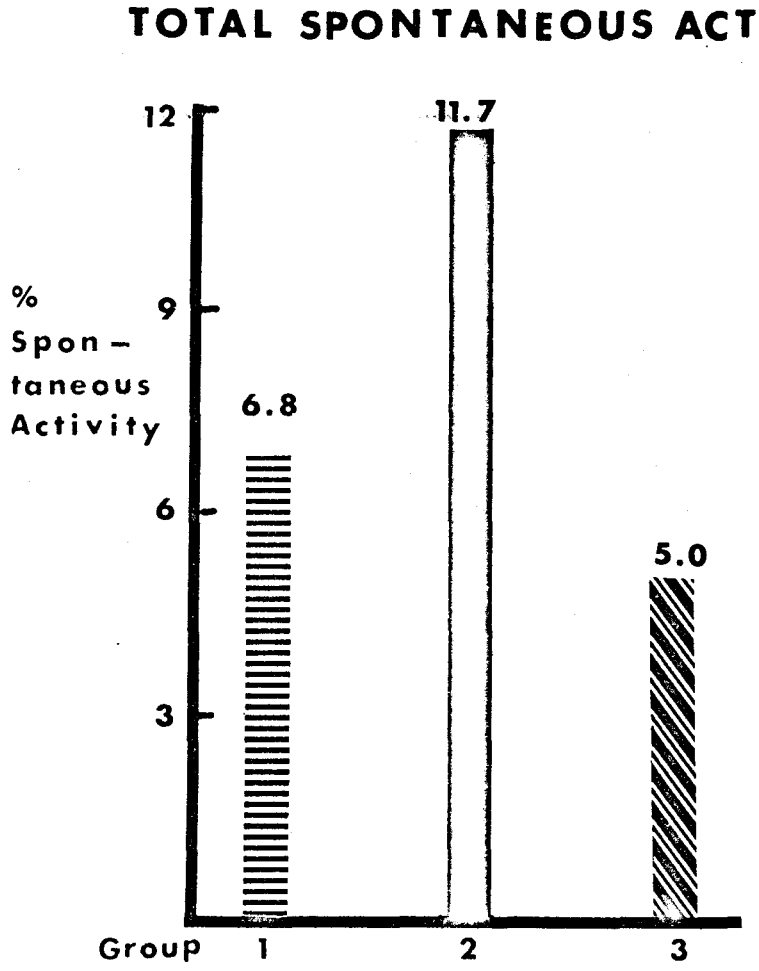

TOTAL SPONTANEOUS ACTIVITY
12
9
6
3
% Spon – taneous Activity
6.8
11.7
5.0
Group
1
2
3

FIGURE 25

LEGEND FOR FIGURE 25.

The percent of trials on which spontaneous activity occurred during the entire experiment is depicted as the ordinate. The appropriate group is depicted as the abscissa. Group II, the "no CS" group, had the greatest frequency of occurrence of spontaneous activity. Spontaneous activity is used synonymously with spontaneous crossings.

TABLE 4

CHI SQUARE ANALYSIS FOR GROUP DIFFERENCES IN SPONTANEOUS ACTIVITY*

GROUP	NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS OBSERVED		NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS ABSENT		TOTALS
	OBS.	EXP.	OBS.	EXP.	
I	19	(21.94)	260	(259.06)	279
II	33	(22.18)	249	(259.82)	282
III	14	(21.88)	264	(256.12)	278
TOTALS	66		773		839

LEGEND FOR TABLE 4

The null hypothesis is that no real difference in the frequency of occurrence of SA exists between the groups; that is, the frequency of occurrence of SA is independent of group classification. The value for Chi Square is 8.207 and was calculated with the continuity correction. There are two degrees of freedom and "p" is < 0.05. Therefore, the null hypothesis is rejected and we have evidence that a real difference exists in the frequency of occurrence of spontaneous activity, and is dependent upon the difference in the groups. The x^2 test is used as a 2 x n table with occurrence and non-occurrence data as described in Batson (1960) p.40. Spontaneous activity refers to spontaneous crossing. OBS. = observed and EXP. = expected.

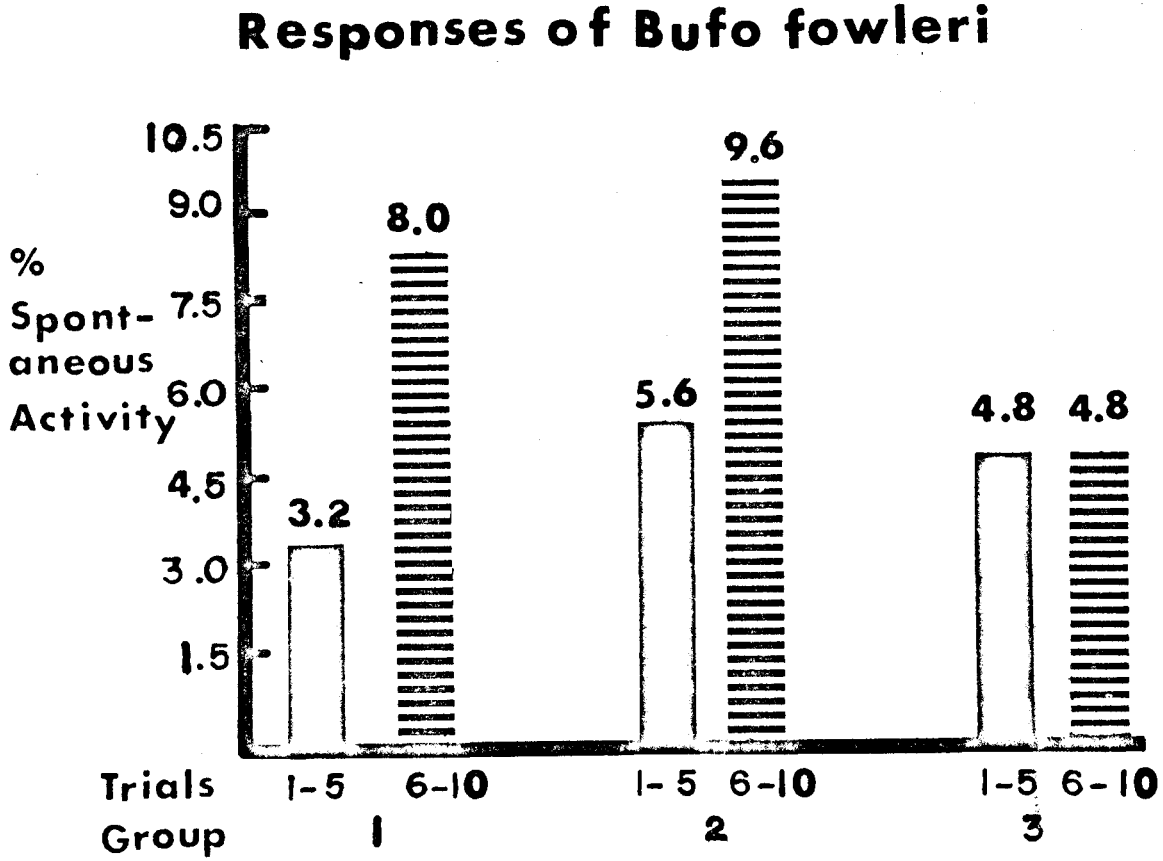

Responses of Bufo fowleri
10.5
9.0
7.5
6.0
4.5
3.0
1.5
% Spont-aneous Activity
3.2
8.0
5.6
9.6
4.8
4.8
Trials
1-5
6-10
1-5
6-10
1-5
6-10
Group
1
2
3

FIGURE 26

LEGEND FOR FIGURE 26.

The percent of spontaneous activity observed for trials one thru five and trials six thru ten are plotted as the ordinate. The respective trial blocks and groups are arranged along the abscissa. Group 1, GI, had the CS overhead in the safe chamber. Group 2, GII, had no CS. Group 3, GIII, had the CS in the safe chamber flashing at twice a second. The Binomial t-test was used to compare the significance of any difference observed within a group. Both Groups I and II showed a greater level of SA in the later five trials than in the first five trials. In Group III no observable change is seen. * = $p<.05$ one tailed Binomial t-test; N = 250 observations per five trials; t = 1.66; d.f. $=\infty$; p(1-5) = 0.032; p(6-10) = 0.086. This figure represents data for Experiment 8. Spontaneous activity is used synonymously with spontaneous crossings.

Figure 27 depicts the results of Bufo fowleri in experiment 9. These results are plotted as the average exit time for each consecutive trial. One can see that a great deal of trial to trial variability occurred, and that no consistent trend towards a steady decrease in exit times is observable. Similarly, Figure 28 depicts an analagous situation for Bufo americanus. A linear regression analysis of the mean exit time versus trial number was made via the least squares method for the "overhead CS" group and the "no CS" group. The Pearson Correlation Coefficients indicate that there was no reason to expect the exit time values to be associated in any linear way with trial numbers for either of the two groups: $r = -0.1277$ for the "overhead CS" group and $r = 0.2203$ for the "no CS" group.

Moreover, for both Bufo fowleri and Bufo americanus (Table 5) there are no statistically significant differences between the average exit time per group for the experiment. However, some significant changes did occur when the first five trials were compared to the last five training trials. For the "overhead CS" group of Bufo fowleri, trials six thru ten had a larger mean exit time than trials one thru five. For the "no CS" group and the "flashing CS" group, there was no statistical significance to the differences between the average exit times for the first and last five trials; see Table 6. Similarly, for Bufo americanus the higher mean exit time observed for trials six thru ten versus trials one thru five was statistically signifi-

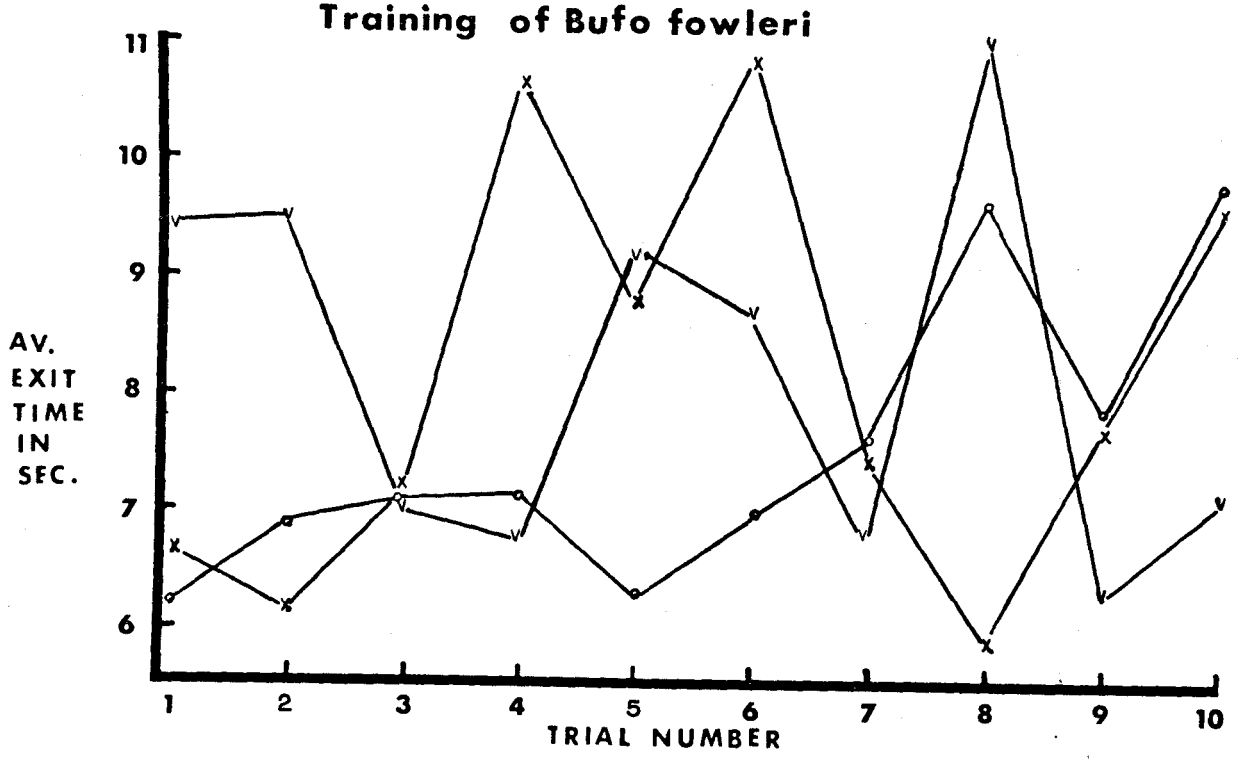

Training of Bufo fowleri
AV. EXIT TIME IN SEC.
11
10
9
8
7
6
1
2
3
4
5
6
7
8
9
10
TRIAL NUMBER

FIGURE 27

LEGEND FOR FIGURE 27

The average exit time per trial number for each group is plotted as the ordinate. The corresponding trial numbers are plotted on the abscissa. Only Bufo fowleri are represented. Data are from Experiment 9. Group I, GI, which is plotted as (O—O) received CS overhead in the safe chamber. Group II, GII, which is plotted as (v—v) received no CS for a period of three seconds prior to shock. Group III, GIII, which is plotted as (x—x) received the CS as a flashing light at eye level in the safe chamber. The intertrial interval was one minute. CS or "no CS" occurred for three seconds prior to shock onset. Both the CS and UCS followed either the CS alone or "no CS" period and continued until the animal escaped. The interior side walls and center of the chamber were covered with black construction paper. The interior of the far walls were covered with blue construction paper. The room lights were off during training. The wide variation in average exit times can readily be seen in the figure. No general trends can be discerned for either Group II or Group III. However, a slight tendency towards increased average exit time values appears to occur in Group I.

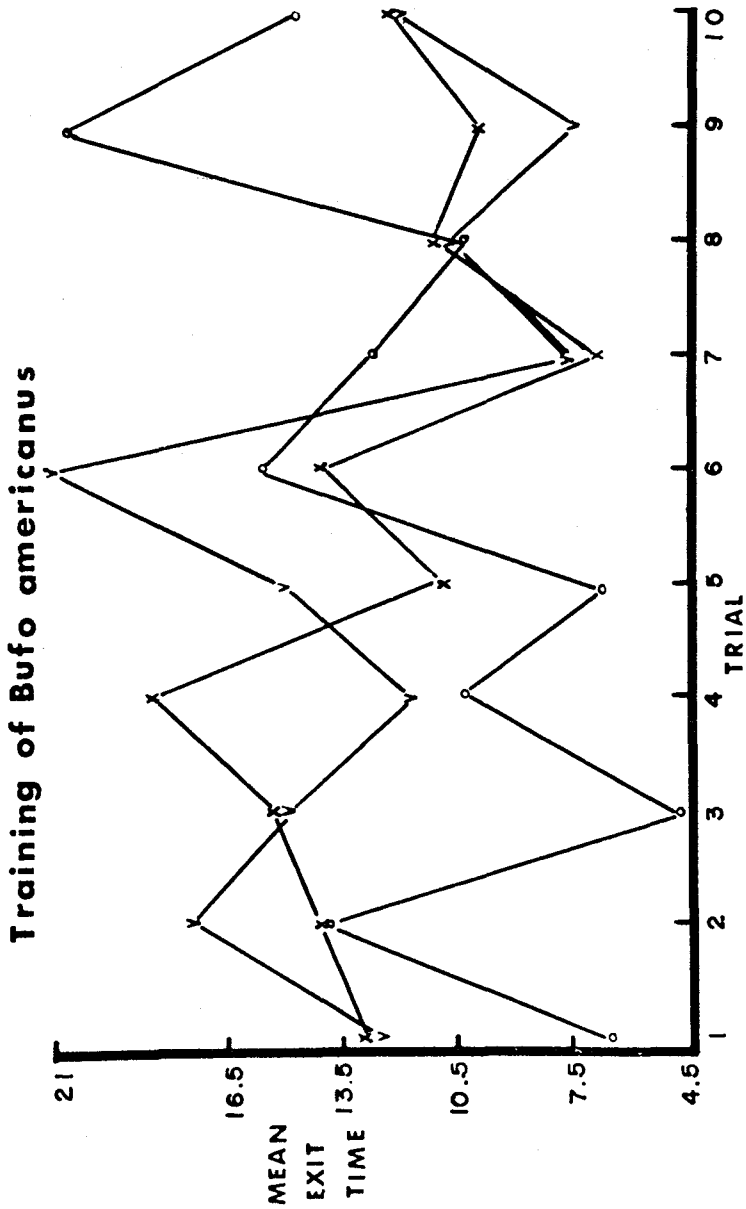

Training of Bufo americanus
MEAN EXIT TIME
21
16.5
13.5
10.5
7.5
4.5
1
2
3
4
5
6
7
8
9
10
TRIAL

FIGURE 28

LEGEND FOR FIGURE 28

The results depicted in this figure are for Experiment 9 and deal with Bufo americanus. The ordinate is the average exit time per trial number. The corresponding trial number is plotted along the abscissa. Each group is represented separately. Group I, GI, which is plotted as (O—O) had the CS appear overhead in the safe chamber. Group II, GII, which is plotted as (v—v) had no CS presented during the normal period of three seconds prior to shock onset. Group III, GIII, which is plotted as (x—x) had the CS appear in the safe chamber at eye level and it was flashing twice a second. Some trends are suggested by the figure. Group I's exit times seem to increase along with experience. Meanwhile both Group II and Group III's values tend to decrease with progressive experience. Two trends were analysed for closeness of fit to the linear regression equation by the least squares method. Group I had a line with positive slope and Group II had a line with negative slope. Neither one, however, had sufficient correlation coefficients to exclude chance. Group I had $r = -0.1277$ while Group II had $r = +0.2203$.

TABLE 5

COMPARISON OF MEAN EXIT TIMES THROUGHOUT THE ENTIRE EXPERIMENT FOR BUFO FOWLERI AND BUFO AMERICANUS

COMPARISON	GROUP	NO. OF ANIMALS	NO. OF VALUES	MEAN EXIT TIME	STANDARD DEVIATION OF MEAN	DIFFERENCE	t	d.f.	p	SIG
A	GI	3	151	7.42	4.10	.08	0.1330	300	<.5	N.S.
	GII	3	151	7.50	5.48					
B	GI	3	151	7.42	4.10	.04	0.0703	301	<.5	N.S.
	GIII	3	152	7.38	4.69					
C	GII	3	151	7.50	5.48	.12	0.1878	301	<.5	N.S.
	GIII	3	152	7.38	4.69					
D	GI	2	97	12.26	13.97	1.34	0.16	152	<.5	N.S.
	GII	2	57	13.60	12.19					
E	GI	2	97	12.26	13.97	0.42	0.24	197	<.5	N.S.
	GIII	2	102	11.84	10.29					
F	GII	2	57	13.60	12.19	1.76	0.97	157	<.5	N.S.
	GIII	2	102	11.84	10.29					

LEGEND FOR TABLE 5

This table deals with the results of Experiment 9. Comparisons A, B, and C deal only with Bufo fowleri, while Comparisons D, E, and F deal with Bufo americanus. The null hypothesis for each comparison is that no real difference in the mean exit times existed between any two groups. In all cases the null hypothesis is accepted, because a significant probability exists that the observed differences are due to chance alone.

cant for the "overhead CS" group, while no statistical significance was associated with the other comparisons; see Table 6.

Analysis of spontaneous crossings for this experiment revealed the following. A combined species and procedure difference was observed; see Figure 29. Table 7 and Table 8 separate the species and examine the procedures for differences. Both tables reveal differences which have $<.05$ probability of occurrence by chance alone. This suggests that within each group there is a difference due to the procedures.

The day to day comparisons of the occurrence of spontaneous crossings do not show consistent trends. However, in the "no CS" procedure BF showed an increase in spontaneous crossings the last two days of the experiment, $p<.01$. In addition the decrease in spontaneous crossings for the flashing CS group, $p<.05$, was statistically significant, while the decrease in the overhead CS group was not significant; see Fig 29 and Table 7. Furthermore, a significant increase in spontaneous crossings occurred in the Bufo americanus group with no CS, $p<.05$, while the changes in the other groups were not significant; see Fig 29 and Table 8.

The species Bufo cognatus became available and was trained in exactly the same fashion as the Bufo fowleri and Bufo americanus species. Figure 30 illustrates the high degree of variability in the mean exit times when they are compared on a trial by trial basis. Figure 30 also indicates a slight reduction in variability of the mean exit time values in the last

TABLE 6

COMPARISON OF MEAN EXIT TIMES FOR TRIAL 1-5 AND 6-10 IN BUFO FOWLERI AND BUFO AMERICANUS

COMPARISON	GROUP		NO. OF ANIMALS	NO. OF VALUES	MEAN EXIT TIME	STANDARD DEVIATION OF MEAN	DIFFERENCE*	t	d.f.	p	SIG.
A	GI	1-5	3	68	6.73	2.74	1.40	2.05	135	< .05	Sig.
	GI	6-10	3	69	8.13	4.95					
B	GII	1-5	3	66	7.77	6.90	.21	0.21	123	>.05	N.S.
	GII	6-10	3	59	7.98	4.39					
C	GIII	1-5	3	63	7.54	4.93	.35	0.42	129	>.05	N.S.
	GIII	6-10	3	68	7.19	4.73					
D	GI	1-5	2	32	8.68	9.98	6.76	2.00	67	< .05	Sig.
	GI	6-10	2	37	15.44	16.70					
E	GII	1-5	2	27	14.73	13.32	2.21	0.62	48	> .05	N.S.
	GII	6-10	2	23	12.52	11.69					
F	GIII	1-5	2	44	14.14	12.81	3.28	1.36	84	> .05	N.S.
	GIII	6-10	2	42	10.96	8.27					

* Unpaired student t-test 2 tailed

** In seconds

LEGEND FOR TABLE 6

This table illustrates the results of Experiment 9. Comparisons A, B, and C are for Bufo fowleri. Comparisons D, E, and F deal with Bufo americanus. The null hypothesis is that there is no significant difference between the last five trials and the first five trials. The null hypothesis is rejected only for the overhead CS, Group I, for both species. See Appendix B for details of the procedure.

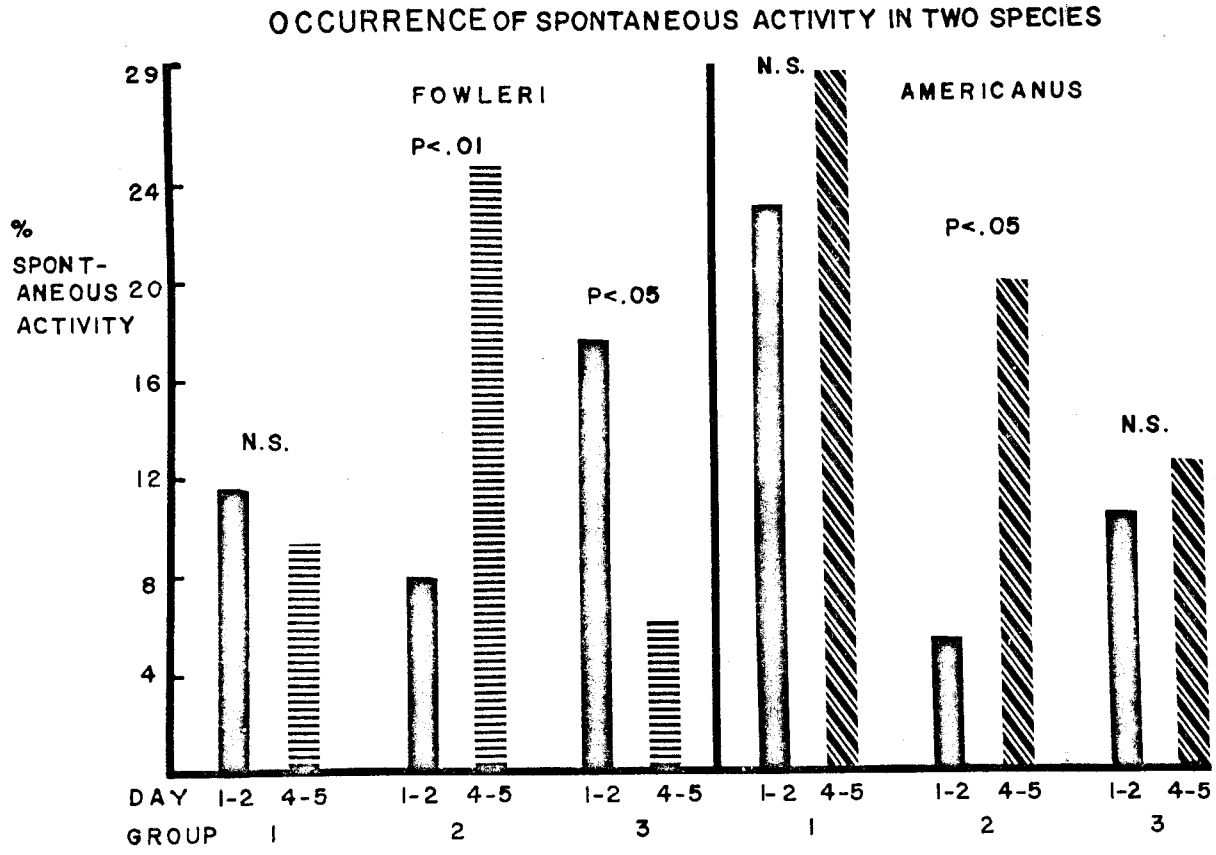

OCCURRENCE OF SPONTANEOUS ACTIVITY IN TWO SPECIES
%
SPONT-
ANEOUS
ACTIVITY
29
24
20
16
12
8
4
FOWLERI
P<.01
P<.05
N.S.
N.S.
AMERICANUS
P<.05
N.S.
DAY 1-2 4-5 1-2 4-5 1-2 4-5 1-2 4-5 1-2 4-5 1-2 4-5
GROUP 1 2 3 1 2 3

FIGURE 29

LEGEND FOR FIGURE 29

This figure represents the results of Experiment 9. The ordinate is the percent of spontaneous activity observed in the experiment. The abscissa consists of the appropriate groups. The left part of the figure depicts the results of Bufo fowleri. The right half of the figure depicts the results of Bufo americanus. The training procedures are outlined in Appendix A. The Binomial t-test was used to test the hypothesis that there was no difference between any group. It can be seen that the only cases where apparent adaptive learning occurred was with the control groups, GII, in both species which showed more spontaneous crossings on days 4 and 5. More spontaneous activity was observed in Bufo americanus compared to Bufo fowleri. Spontaneous activity is used synonymously with spontaneous crossings. See Table 8 for statistical analysis for Bufo americanus and and Table 7 for statistical analysis for Bufo fowleri.

TABLE 7

SPONTANEOUS CROSSING DATA FOR BUFO FOWLERI *

GROUPS	NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS OBSERVED		NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS ABSENT		TOTALS
	OBS.	EXP.	OBS.	EXP.	
I	15	(22.27)	152	(144.73)	167
II	36	(24.93)	151	(162.07)	187
III	19	(22.80)	151	(148.2)	171
TOTALS	70		455		525

* Chi Square analysis: two degrees of freedom, $x^2 = 9.1409$, $p<0.05$

LEGEND FOR TABLE 7

This table represents the results of Experiment 9. It is a Chi Squared analysis on the frequency of occurrence of spontaneous crossings for Bufo fowleri observed during the experiment. The null hypothesis is that there is no real difference between the frequency of occurrence of spontaneous activity among the three procedures. Chi Square value of 9.14 with two degrees of freedom gives a less than 5% probability that a distribution such as this would be do to chance alone. Therefore, the null hypothesis is rejected, and one can conclude that there is evidence for a significant difference in the spontaneous activity seen under the different procedures. Spontaneous activity is synonymous with spontaneous crossings.

TABLE 8

SPONTANEOUS CROSSING DATA FOR BUFO AMERICANUS

GROUPS	NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS OBSERVED		NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS ABSENT		TOTALS
	OBS.	EXP.	OBS.	EXP.	
I	32	(22.18)	106	(115.82)	138
II	7	(12.05)	68	(62.95)	75
III	15	(19.77)	108	(103.23)	123
TOTALS	54		282		336

* Chi Squared analysis: two degrees of freedom, $x^2 = 9.0731$, $p < 0.05$

LEGEND FOR TABLE 8

This table represents the results of Experiment 9. It is a Chi Squared analysis of the frequency of occurrence of spontaneous crossings for Bufo americanus. The null hypothesis was that no real difference existed between the observed frequencies of occurrence of spontaneous activity among the different procedures. A x^2 value as high as 9.07 with two degrees of freedom gave a less than 5% probability that a distribution such as it could occur by chance alone. Therefore, the null hypothesis was rejected, and one can conclude that there is evidence for a significant difference in the spontaneous activity seen under the different procedures. Spontaneous activity refers to spontaneous crossings.

four trials.

The mean exit times per trial were examined to see if the groups differed in any significant fashion. Table 9 illustrates the comparisons. Group II, the "no CS" group, had the lowest mean exit time of 6.24 seconds. Group III, the "flashing CS" group, had an intermediate mean exit time of 6.67 seconds. Group I, the "overhead CS" group, had the highest mean exit time of 7.85 seconds. The observed differences in mean exit times between Group I versus Group II, $t = 3.266$ and $p<0.001$, and Group I versus Group III, $t = 2.7119$ and $p<.005$, were statistically significant.

Further analysis of the mean exit time values is depicted in Table 10. In this table the mean exit time for trials six thru ten is compared to the mean exit time for trials one thru five. A decrease in the mean exit time value for trials six thru ten versus trials one thru five is considered to be the result of adaptive learning. Group I, the "overhead CS" group, had the slowest overall mean exit time and was the only group to exhibit learning by this method of analysis. Group III, the "flashing CS" group, also exhibited a decreased mean exit time for trials six thru ten. However, this mean was not significantly different from the mean exit time value for trials one thru five. Although Group II, the "no CS" group, displayed the shortest overall mean exit time, it was the only group to exhibit the usual response of an increased mean exit time for trials

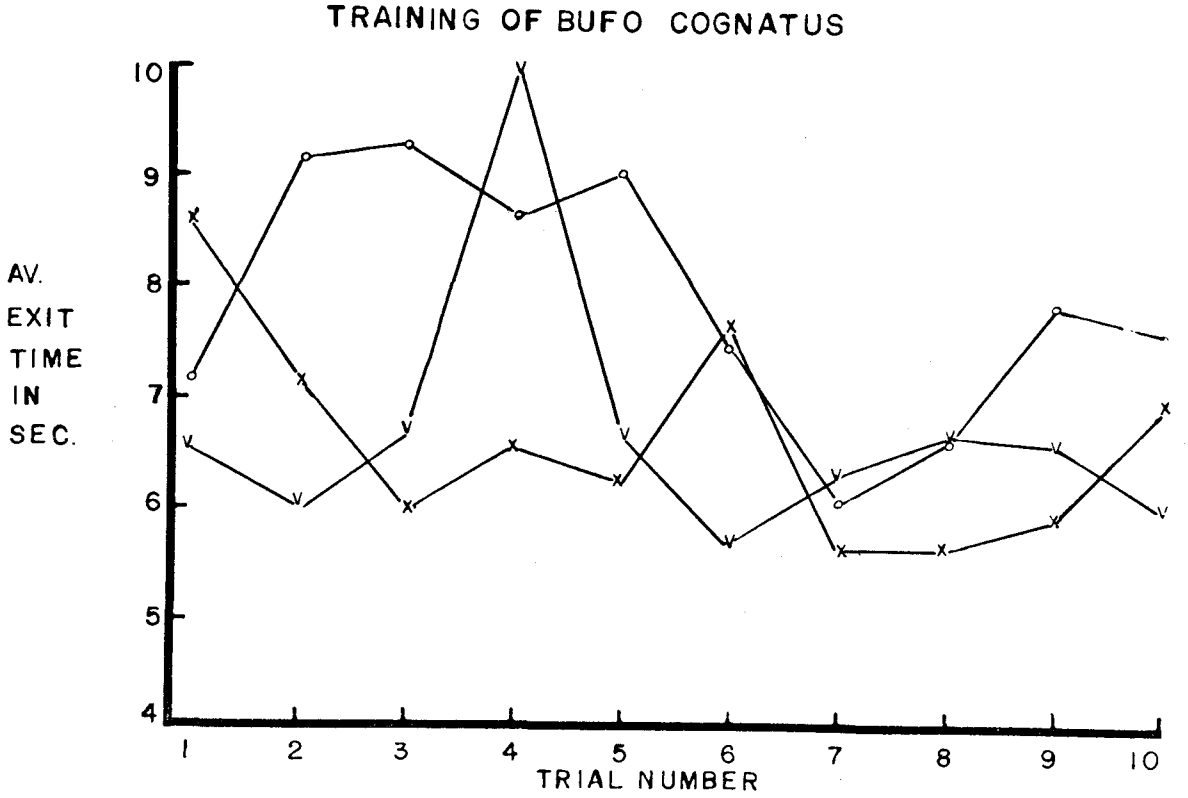


FIGURE 30. This figure represents the results observed in Experiment 10. Bufo cognatus was the species used. The ordinate is the average exit time per trial number. The abscissa is the corresponding trial number. The procedure is outlined in Appendix B. It can be seen that all three groups have a tendency towards less variability at the end of the training session. The three groups are plotted as follows: GI (°——°) was the "overhead CS" group, GII (v——v) was the "no CS" group, and GIII (x——x) was the "flashing CS" group.

TABLE 9

COMPARISON OF THE MEAN EXIT TIMES FOR BUFO COGNATUS

COMPARISON	GROUP	NO. OF ANIMALS	NO. OF VALUES	MEAN EXIT TIME	STANDARD DEVIATION OF MEAN	DIFFERENCE	t	d.f.	p	SIG
A	GI	3	134	7.85	3.98	1.61	3.27	274	$<.05$	Sig
	GII	3	142	6.24	4.18					
B	GI	3	134	7.85	3.98	1.18	2.71	267	$<.05$	Sig
	GIII	3	135	6.67	3.09					
C	GII	3	142	6.24	4.18	.43	0.96	275	$>.05$	N.S.
	GIII	3	135	6.67	3.09					

LEGEND FOR TABLE 9

This table depicts the results of Experiment 10 conducted with Bufo cognatus. The mean exit time per group is examined. See Appendix B for details about the procedure. The null hypothesis is that no difference exists between the average exit times for the two groups being compared. This hypothesis is accepted only for Comparison C. The other observed differences are statistically significant. GII = no CS; GI = CS overhead in safe chamber; GIII = flashing CS in safe chamber.

TABLE 10

COMPARISON OF FIRST 5 TRIALS AND LAST 5 TRIALS OF BUFO COGNATUS

COMPARISON	GROUP		NO. OF ANIMALS	NO. OF VALUES	MEAN EXIT TIME	STANDARD DEVIATION OF MEAN	DIFFERENCE	t	d.f.	p.	SIG
A	GI	1-5	3	57	8.69	5.11	1.41	1.71	103	< .05	Sig.
	GI	6-10	3	48	7.28	2.84					
B	GII	1-5	3	63	6.00	4.43	.25	.376	116	> .05	N.S.
	GII	6-10	3	55	6.25	2.5					
C	GIII	1-5	3	63	6.89	3.35	.51	.922	122	> .05	N.S.
	GIII	6-10	3	61	6.38	2.71					

LEGEND FOR TABLE 10

This table represents the data derived from Experiment 10 with Bufo cognatus. All three groups are compared with regard to changes in performance of the first five and the next five trials. The unpaired, one tailed, Student t-test was used. For Group I, the statistically significant decrease in the mean exit time of trials six through ten relative to trials one through five indicates classical avoidance conditioning.

six thru ten.

In addition to the analysis of mean exit time values, an analysis was made of the degree of spontaneous activity observed within the groups. Table 11 depicts a x^2 analysis of the three procedures to test for a statistically significant difference between the relative frequencies of occurrence of spontaneous activity amongst the three groups. There is evidence for a significant difference with $x^2 = 16.6169$, $p < .01$ and two degrees of freedom.

Table 12 depicts a comparison between the degree of spontaneous activity observed on days one and two of training compared to days four and five of training. Only the "flashing CS" group had a significant difference between the amount of spontaneous activity during the first and last days of training, $t = 2.9834$, Binomial t-test, ∞ d.f.

Another modification was made in the conditioning apparatus by incorporating a simple contrast into the design. The interior walls and center were now lined with white construction paper and the far wall with black. This simple black and white contrast was to replace the previous blue-black contrast. The contrast of white against black is created by looking through the escape route towards the far wall. This contrast is made by the center walls being white and the far walls being black. Five toads of the Bufo fowleri species were included in each group. More details about the procedure are in Appendix B.

TABLE 11

GROUP	NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS OBSERVED		NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS ABSENT		TOTALS
	OBS.	EXP.	OBS.	EXP.	
I	27	(32.18)	155	(149.82)	182
II	49	(32.53)	135	(151.47)	184
III	17	(28.29)	143	(131.72)	160
TOTALS	93		433		526

LEGEND FOR TABLE 11

This table depicts the results of Experiment 10 with Bufo cognatus. The value of Chi Square is 16.6150 which under a two tailed analysis with two degrees of freedom results in a p value less than 0.01. With a value of Chi Square this high, one has evidence that there is a significant difference between the frequency of occurrence of spontaneous activity between the groups with only a 1% probability that it is due to chance alone. Spontaneous activity is synonymous with spontaneous crossings. GI had the CS overhead in the safe chamber. GII had no CS. GIII had the flashing CS in the safe chamber.

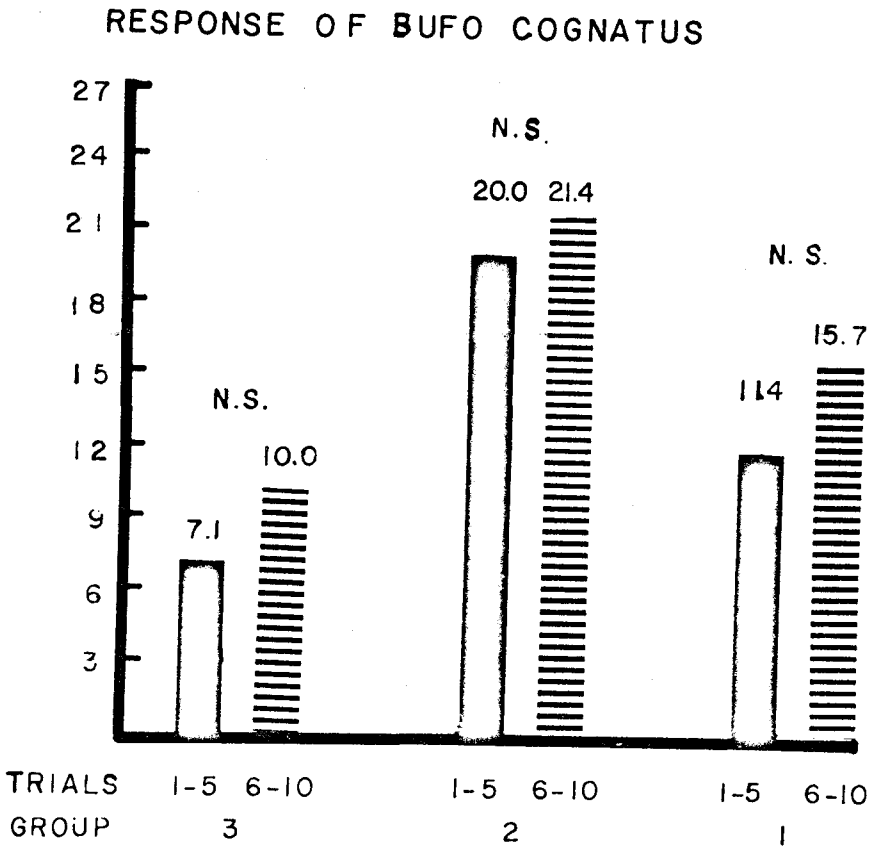


FIGURE 31. The percent of spontaneous activity in a block of five trials is plotted as the ordinate. The trial blocks one thru five and six thru ten are plotted along the abscissa. Results are for Experiment 10 with Bufo cognatus. The Binomial "t" test was used to test for statistically significant differences in the intra group values. Group I had an overhead CS in the safe chamber. Group II had no CS. Group III had the flashing CS in the safe chamber. Details of the procedure are given in Appendix B. A greater increase in the percent of spontaneous activity occurred in those groups which were trained with a CS. Spontaneous activity is synonymous with spontaneous crossings.

TABLE 12

COMPARISON OF FREQUENCY OF OCCURRENCE OF SPONTANEOUS ACTIVITY ON DAYS 1 AND 2 VERSUS DAYS 4 AND 5

GROUP	DAYS	NO. OF SPONTANEOUS CROSSES	NO. OF TRIALS	p	q	t	P VALUE
III	1 & 2	3	65	0.046	0.954	2.98	<0.01
	4 & 5	14	64	0.219	0.781		
II	1 & 2	17	78	0.218	0.782	0.60	N.S.
	4 & 5	11	62	0.177	0.822		
I	1 & 2	13	78	0.167	0.833	0.08	N.S.
	4 & 5	11	68	0.167	0.838		

* Two tailed Binomial t-test. P is the probability of occurrence and q is the probability of nonoccurrence of spontaneous activity.

LEGEND FOR TABLE 12

In this table the spontaneous activity which occurred on the first two days of training is compared to that which occurred on the last two days of training. Group III was the flashing CS group. Groups II had no CS. Group I had the overhead CS in the safe chamber. The frequency of occurrence of spontaneous activity on days one and two is compared to that which occurred on days four and five for each group via the Binomial t-test. Note, that only in the flashing CS group is there a significant difference between the first two and the last two days of training. The observed direction of change indicates that adaptive learning occurred in this group. Spontaneous activity refers to spontaneous crossing.

As can be observed in Figure 32, the exit times appear to be less variable than those which were formerly seen in other experiments. Furthermore, the "overhead CS" group which had the CS in the safe chamber, as expected, showed the best response. The group with the "no CS" procedure showed slightly higher values while the group with the "overhead CS" in the shocking chamber had the highest mean exit time values. A slight tendency is still evident in these groups towards longer exit times in the later training trials. The observed differences in exit times are substantiated quantitatively in Table 13 and Figure 33. The "overhead CS" in shocking chamber group had the highest exit times and these were significantly different from both the "no CS" group ($t = 3.1325$, $p < .005$, one tailed test, 496 d.f.), and from the "overhead CS" in safe chamber group ($t = 5.2569$, $p < .005$, one tailed, 499 d.f.). The differences between the "no CS" group and the "CS overhead in safe chamber group" is also significant at the $p < .01$ level, two tailed, $t = 2.3815$, 499 d.f.

However, the difference seen in Figure 34 which compares trials one thru five to trials six thru ten for each group just borders on being statistically significant; see Table 14.

Figure 35 indicates the total level of spontaneous crossing in each group. Table 15 quantitates this difference as being statistically significant, $p < .01$. Figure 36 indicates the different degrees of spontaneous crossings exhibited in trials

PERFORMANCE OF BUFO FOWLERI IN THREE PROCEDURES

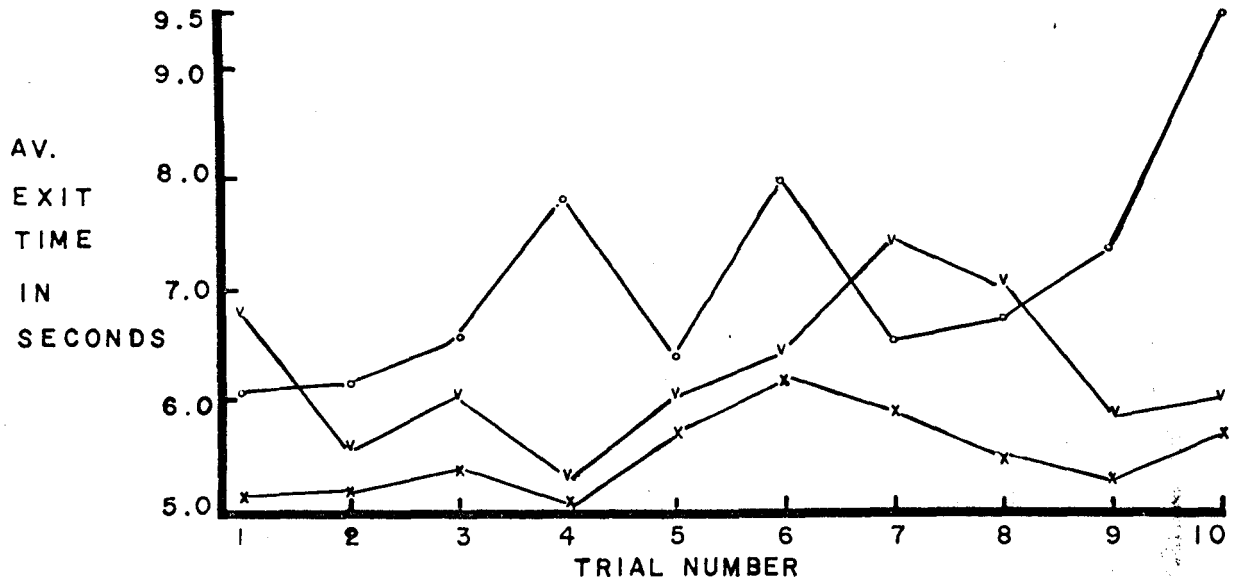


FIGURE 32

LEGEND FOR FIGURE 32.

This figure represents the results of Experiment 11. The ordinate is the average exit time per numbered trial. The abscissa is the corresponding trial number. The results are for Bufo fowleri. The procedure is detailed in Appendix B. The group indicates a very slight increase in mean exit times occurring later in the training session. The variability in trial to trial responses appears to be somewhat reduced by comparison to previous studies. The following symbols represent the different groups: GIV (°——°) equals the overhead group CS in shocking chamber; GVI (v—v) equals no CS; GV (x——x) equals the overhead CS in the safe chamber.

TABLE 13

COMPARISONS OF GROUP MEAN EXIT TIME*

COMPARISON	GROUP	NO. OF ANIMALS	NO. OF OBSER-VATIONS	MEAN EXIT TIME	STANDARD DEVIATION OF MEAN	DIF-FER-ENCE	t	d.f.	p	SIG
GIV vs GVI	GIV	5	249	7.17	4.03	0.99	3.1325	496	<.005	Sig
	GVI	5	249	6.18	2.86					
GIV vs GV	GIV	5	249	7.17	4.04	1.53	5.2669	499	<.005	Sig
	GV	5	252	5.64	2.22					
GVI vs GV	GVI	5	249	6.18	2.86	.54	2.38	499	<0.01	Sig
	GV	5	252	5.64	2.22					

* Unpaired one tailed student t test

LEGEND FOR TABLE 13

A summary of total mean exit times for Experiment 11 with Bufo fowleri. All differences were significant, unpaired 2 tailed, Student t-test. Group V the overhead CS in the safe chamber group had the fastest mean exit time, 5.64 seconds. Group VI with no CS was the next fastest with a mean exit time of 6.18 seconds. While Group IV with an overhead CS in the shocking chamber had the slowest mean exit time at 7.17 seconds.

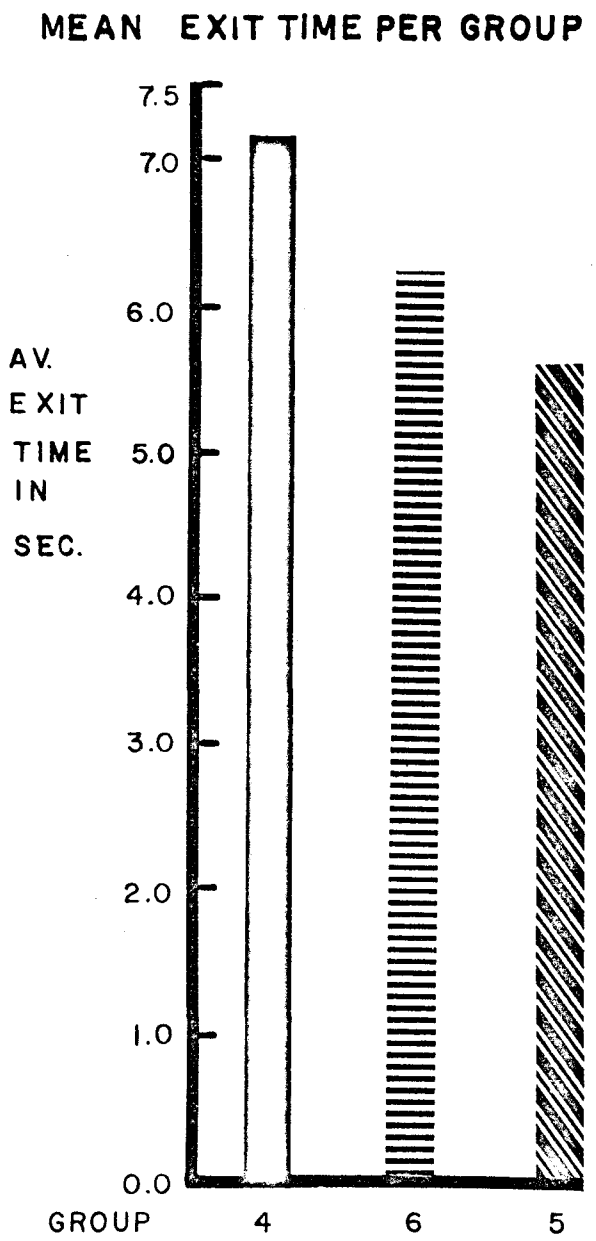

MEAN EXIT TIME PER GROUP
7.5
7.0
6.0
AV.
EXIT
TIME
IN
SEC.
5.0
4.0
3.0
2.0
1.0
0.0
GROUP
4
6
5

FIGURE 33

LEGEND FOR FIGURE 33.

The average exit time per trial is plotted as the ordinate. This value was computed using all of the good trials in the experiment. The abscissa is comprised of the corresponding groups. Group IV had the overhead CS in the shocking chamber. Group VI had no CS, while Group V had the overhead CS in the safe chamber. It can be seen that the slowest group was GIV, followed by GVI and GV. See Table 13 for statistics.

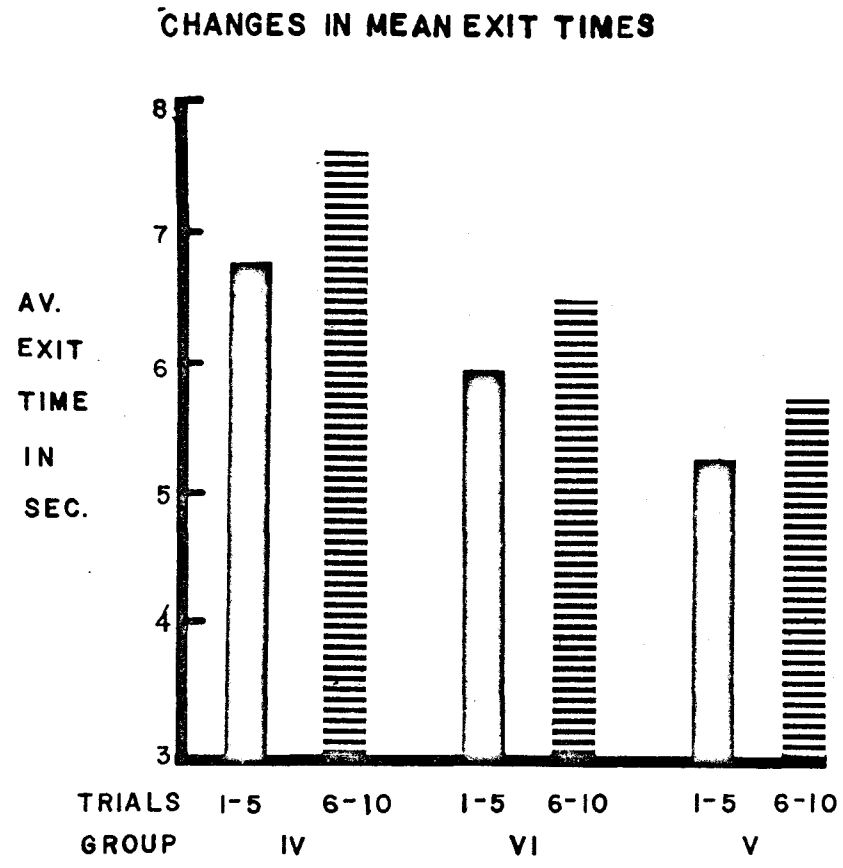


FIGURE 34. This figure represents the results of Experiment 11. The ordinate is the mean exit time for all experimental trials from one thru five and trials six thru ten. The abscissa indicates the experimental group and trial block. Group IV had the CS overhead in the shocking chamber. Group VI had no CS. Group V had the CS overhead in the safe chamber. See Table 14 for statistics.

TABLE 14

COMPARISON OF GROUP MEAN EXIT TIMES FOR TRIALS 1-5 AND TRIALS 6-10 FOR BUFO FOWLERI

COMPARISON	GROUP	NO. OF ANIMALS	NO. OF OBSER-VATIONS	MEAN EXIT TIME	STANDARD DEVIATION OF MEAN	DIF-FER-ENCE	t	d.f.	p	SIG
t 1-5	IV	5	114	6.76	2.38	0.92	1.60	232	<.1	Sig
t 6-10		5	120	7.68	5.24					
t 1-5	VI	5	119	5.93	2.22	0.58	1.52	232	<.1	Sig
t 6-10		5	115	6.51	3.48					
t 1-5	V	5	114	5.29	1.90	0.45	1.57	213	<.1	Sig
t 6-10		5	101	5.74	2.27					

* See legend

LEGEND FOR TABLE 14

The unpaired student t statistic is used to test the one tailed null hypothesis that the observed increase in mean exit times within each group for trials six through ten is not significantly different from the lower means observed for trials one through five. The p values border on significance with $p < 0.1$ in each group. These will be considered biologically important because of the uniformity of data and previous experience. Group IV had the CS overhead in the shocking chamber. Group VI had no CS and Group V had the CS overhead in the safe chamber. See Figure 34.

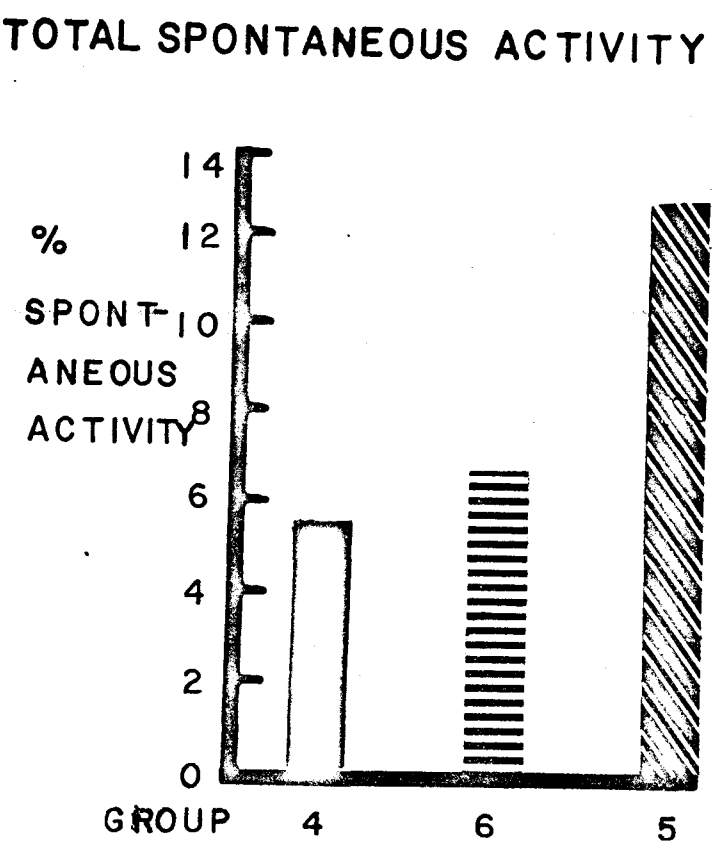


FIGURE 35. This figure depicts the spontaneous activity observed in each group for Experiment 22. The ordinate is the observed percent of trials in which spontaneous activity occurred. The abscissa indicates the corresponding groups. Group IV had the CS overhead in the shocking chamber, Group VI had no CS and Group V had the CS overhead in the safe chamber. Spontaneous activity is synonymous with spontaneous crossing. See Table 15 for statistics.

one thru five and six thru ten for each group. Table 16 indicates that two differences are significant by the one tailed x^2 statistic, those for the CS overhead in shocking chamber and safe chamber groups.

A final modification was made in the training procedure which was the storing of experimental subjects in a controlled environment. A diurnal rhythm was established with a day cycle of fourteen hours duration at a temperature of 23.9° C. and a night cycle of ten hours duration with a temperature of 18.3° C. Animals were housed in this environment for six days prior to training and in between training sessions. The group without any CS was the control group, Group II. Group I had the CS appear overhead in the safe chamber. Group III had the flashing CS in the safe chamber. Groups suffixed with an "A" were trained with the room lights turned on.

Figure 37 shows the performance of Group II, the control group; it displays a general trend for slower exit times and a greater variation in response with progressive experience. As previously mentioned, a classical avoidance response did not occur in any group. Group IIA portrayed on the same figure underwent the same procedure as Group II, but with the room lights on and seemed to be half second faster in exiting than Group II, yet Group IIA still maintained the same general trend towards increasing exit times.

Figure 38 indicates the effect of a CS in Group I with the

TABLE 15

GRAND TOTAL PROCEDURE DIFFERENCE

GROUP	NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS OBSERVED		NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS ABSENT		TOTALS
	OBS.	EXP.	OBS.	EXP.	
IV	15	(22.75)	252	(244.25)	267
VI	18	(22.83)	250	(245.17)	268
V	38	(25.42)	260	(272.58)	298
TOTALS	71		762		833

LEGEND FOR TABLE 15

This table is for the results of Experiment 11. The Chi Squared statistic is used to test whether the observed differences in frequency of occurrence for spontaneous crossings are independent of the grouping. The null hypothesis is that no significant difference in frequency exists. The hypothesis is rejected since the value for Chi Square is 10.809 with two degrees of freedom, and with less than a 1% probability of a value this large being due to chance alone. One therefore has evidence for a real difference in the amount of spontaneous activity between the groups. Group IV had the CS overhead in the shocking chamber. Group VI had no CS. Group V had the CS overhead in the safe chamber. This table is in reference to Fig 35.

TABLE 16

GROUP	NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS OBSERVED		NUMBER OF TRIALS IN WHICH SPONTANEOUS ACTIVITY WAS ABSENT		TOTALS
	OBS.	EXP.	OBS.	EXP.	
IV					
t 1-5	10	(7.0)	115	(118)	125
t 6-10	4	(7.0)	121	(118)	125
TOTALS	14		236		250
p = .0560	d.f. = 1	x^2 = 2.7240	p < .05 SIG.		
VI					
t 1-5	6	(7)	119	(118)	125
t 6-10	8	(7)	117	(118)	125
TOTALS	14		236		250
p = .0560	d.f. = 1	x^2 = 0.3027	p < .5 N.S.		
V					
t 1-5	9	(17)	116	(108)	125
t 6-10	25	(17)	100	(108)	125
TOTALS	34		216		250
p = .1360	d.f. = 1	x^2 = 8.7146	p < .005 SIG.		

LEGEND FOR TABLE 16

This table represents the results of Experiment 11. The Chi Squared analysis is used for each group to see if the observed increase or decrease in frequency of occurrence of spontaneous activity in trials six through ten is significantly different from that which occurred in trials one through five. There is a significant difference in Group IV and Group V. p = probability of occurrence. See Figure 36.

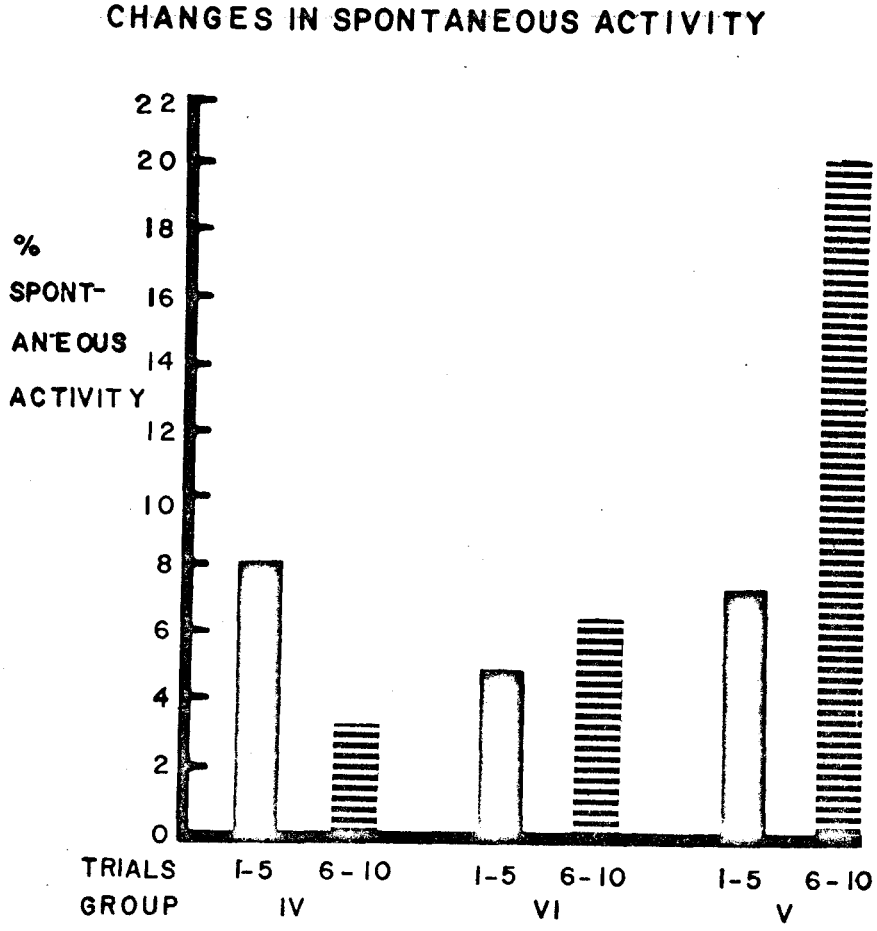

CHANGES IN SPONTANEOUS ACTIVITY
%
SPONT-
ANEOUS
ACTIVITY
22
20
18
16
14
12
10
8
6
4
2
0
TRIALS
GROUP
1-5
6-10
IV
1-5
6-10
VI
1-5
6-10
V

FIGURE 36

LEGEND FOR FIGURE 36.

This figure depicts the difference in spontaneous activity that was observed between trials one thru five and trials six thru ten for each group in Experiment 11. The ordinate is the observed percent of trials in which spontaneous activity occurred. The abscissa indicates the corresponding groups and trial blocks. Group IV had the CS overhead in the shocking chamber. Group VI had no CS. Group V had the CS overhead in the safe chamber. Spontaneous activity is synonymous with spontaneous crossing. See Table 16 for statistics.

CS overhead in the safe chamber. In Group I, the CS may have altered the variability of response from trial to trial and may have altered the trend toward longer exit times as seen in Group II. Group IA however, still shows a variable response under the influence of room lights. One can rationalize this response since the increased ambient illumination effectively reduced the magnitude of change brought about by the small overhead CS light. That is it actively interfered with the perception of the CS.

Figure 39 displays the data summary for Group III and IIIA. The CS may be responsible for the altered response. There was no change in the trend towards longer exit times during the later trials. Group IIIA varied insignificantly from Group III. Thus one may interpret this to mean that the room lights had little effect on the perception of the flashing CS.

Figure 40 compares the level of spontaneous crossings of the first five trials, trials one thru five, to the last five trials, trials six thru ten. It can be readily seen that in all cases (except for the control) the level of spontaneous crossings increased during the second half of the session; proof that these changes are significant is depicted in Table 17.

Figure 41 indicates for all groups that a greater level of spontaneous crossings occurred during the last eight days of training, days nine thru sixteen, which is depicted by the striped bars, than that which occurred during the first eight days of training, days one thru eight, which is depicted by the solid

bars. One may conclude therefore, that there is evidence of an increased frequency of adaptive behavior from day to day as well as within a single training session.

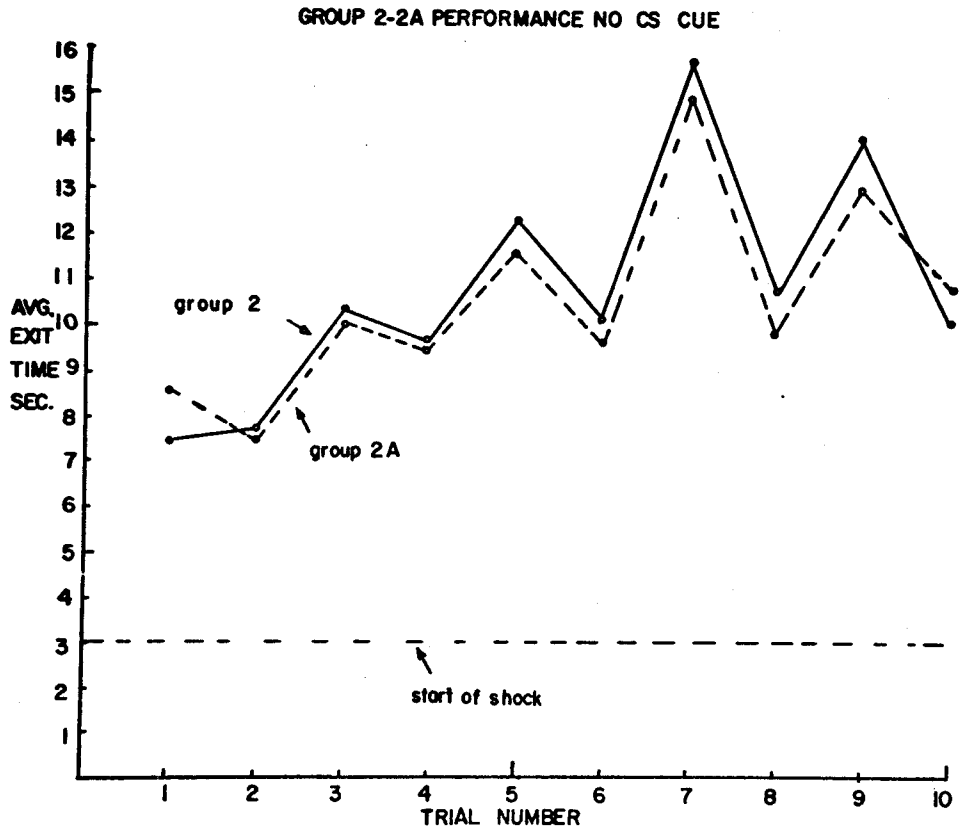


FIGURE 37. This figure depicts the results of Experiment 12. The average exit time per trial number is plotted as the ordinate. The appropriate trial number is plotted as the abscissa. The duration of presentation of CS is three seconds. The dotted line indicates the start of shock. The figure depicts the results of Group II and IIA which had no CS. The "A" suffix indicates that the room lights were on. One can observe that the variability of mean values increases with progressive experience. Furthermore, there seems to be a general tendency towards an increasing mean value with progressive experience.

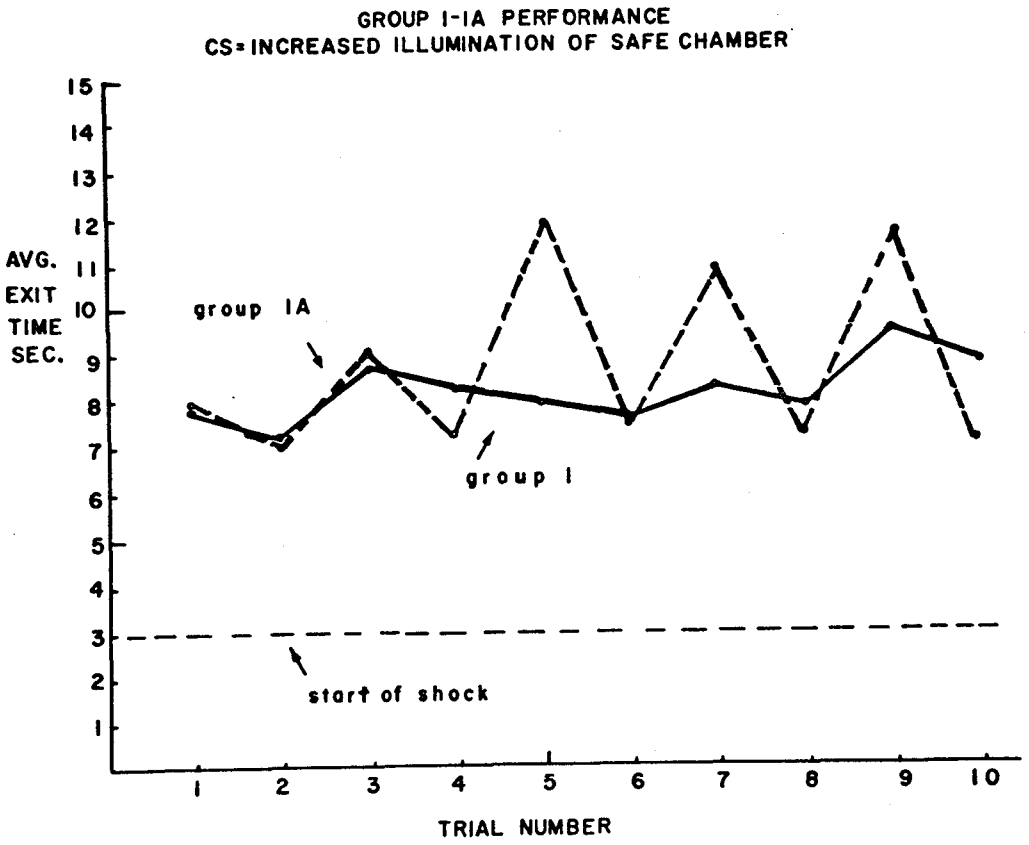

GROUP I-IA PERFORMANCE
CS=INCREASED ILLUMINATION OF SAFE CHAMBER
AVG. EXIT TIME SEC.
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
group IA
group I
start of shock
1
2
3
4
5
6
7
8
9
10
TRIAL NUMBER

FIGURE 38

LEGEND FOR FIGURE 38.

This figure depicts the results of Experiment 12. The average exit time per trial number is plotted as the ordinate. The appropriate trial number is plotted as the abscissa. The duration of presentation of CS is three seconds. The dashed horizontal line indicates the start of shock. The figure depicts the results of two groups, Group I and Group IA, both of which had the CS overhead in the safe chamber. Both groups exhibit a general tendency towards an increasing mean exit time value with progressive experience. One can also observe that the mean exit time values varied greatly in Group IA which was trained with the room lights on. On the other hand, the variability of mean exit times was much less in Group I which was trained with the room lights off. GI (.——.) and GIA (.---.).

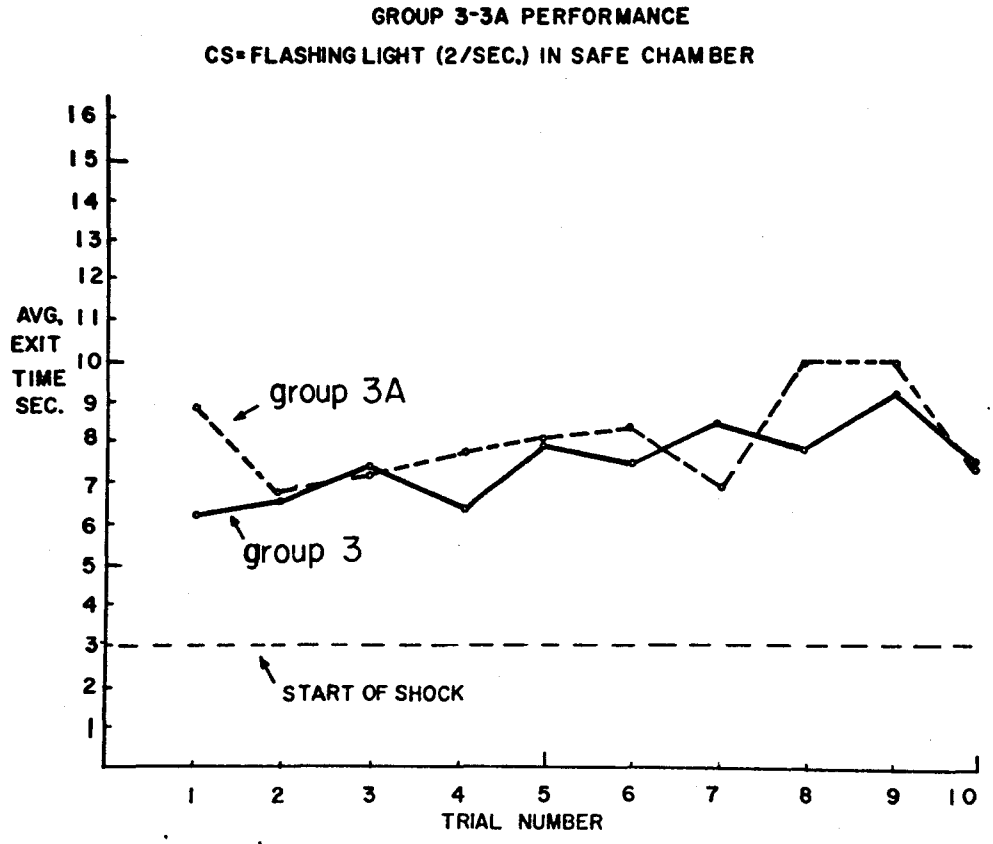

GROUP 3-3A PERFORMANCE
CS= FLASHING LIGHT (2/SEC.) IN SAFE CHAMBER
AVG. EXIT TIME SEC.
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
group 3A
group 3
START OF SHOCK
1
2
3
4
5
6
7
8
9
10
TRIAL NUMBER

FIGURE 39

LEGEND FOR FIGURE 39.

This figure depicts the results of Experiment 12. The average exit time per trial number is plotted as the ordinate. The appropriate trial number is plotted as the abscissa. The duration of presentation of CS is three seconds. The dotted line indicates the start of shock. The figure depicts the results of Group III and Group IIIA which had the CS at eye level in the safe chamber flashing at twice a second. The "A" suffix indicates the room lights were on. One can see that the variability of values seems to remain relatively low. However, a general tendency towards an increasing mean value with progressive experience is obvious in both groups.

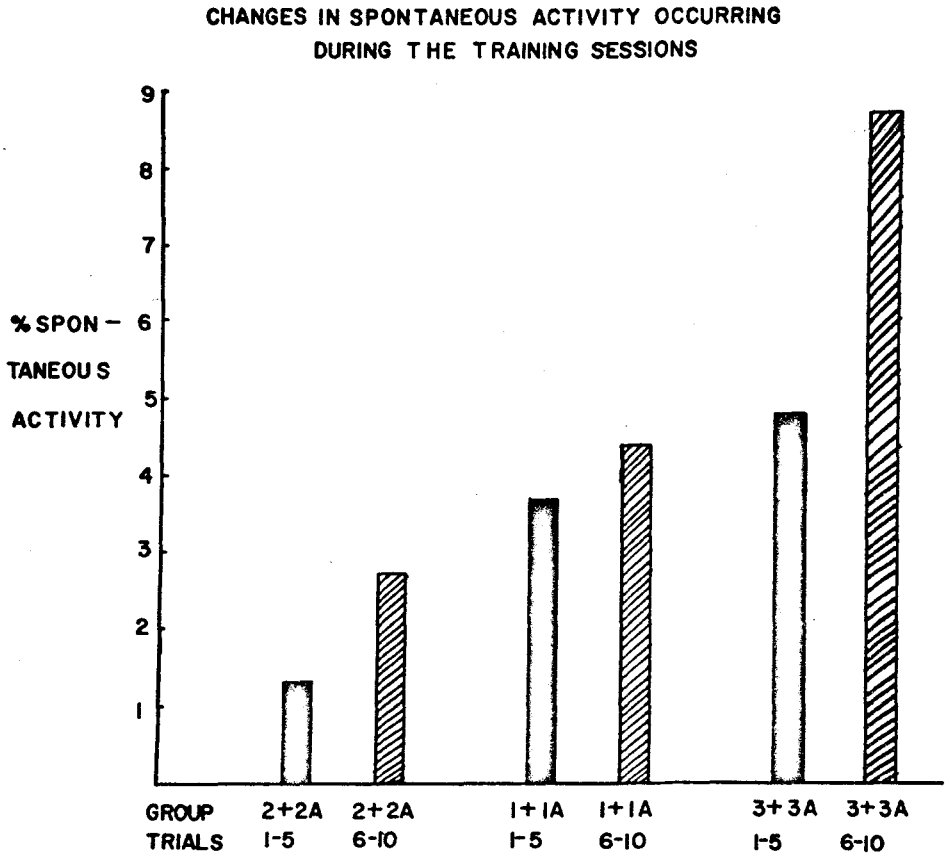

CHANGES IN SPONTANEOUS ACTIVITY OCCURRING
DURING THE TRAINING SESSIONS
% SPON-
TANEOUS
ACTIVITY
9
8
7
6
5
4
3
2
1
GROUP
TRIALS
2+2A
1-5
2+2A
6-10
1+1A
1-5
1+1A
6-10
3+3A
1-5
3+3A
6-10

FIGURE 40

LEGEND FOR FIGURE 40.

This figure depicts the difference in spontaneous activity observed between trials one thru five, solid bars, and trials six thru ten, striped bars, for each group in Experiment 12. Groups with the room lights on were pooled with the others since the changes observed were in the same direction. The abscissa indicates the corresponding groups and trial blocks. Group I and Group IA had the CS overhead in the safe chamber. Group II and Group IIA had no CS cue. Group III and Group IIIA had the CS in the safe chamber flashing at twice at second. The "A" suffix indicates that the room lights were on. One can see that in all cases the level of spontaneous activity increased in the later trials. Spontaneous activity is synonymous with spontaneous crossings. See Table 17 for statistics.

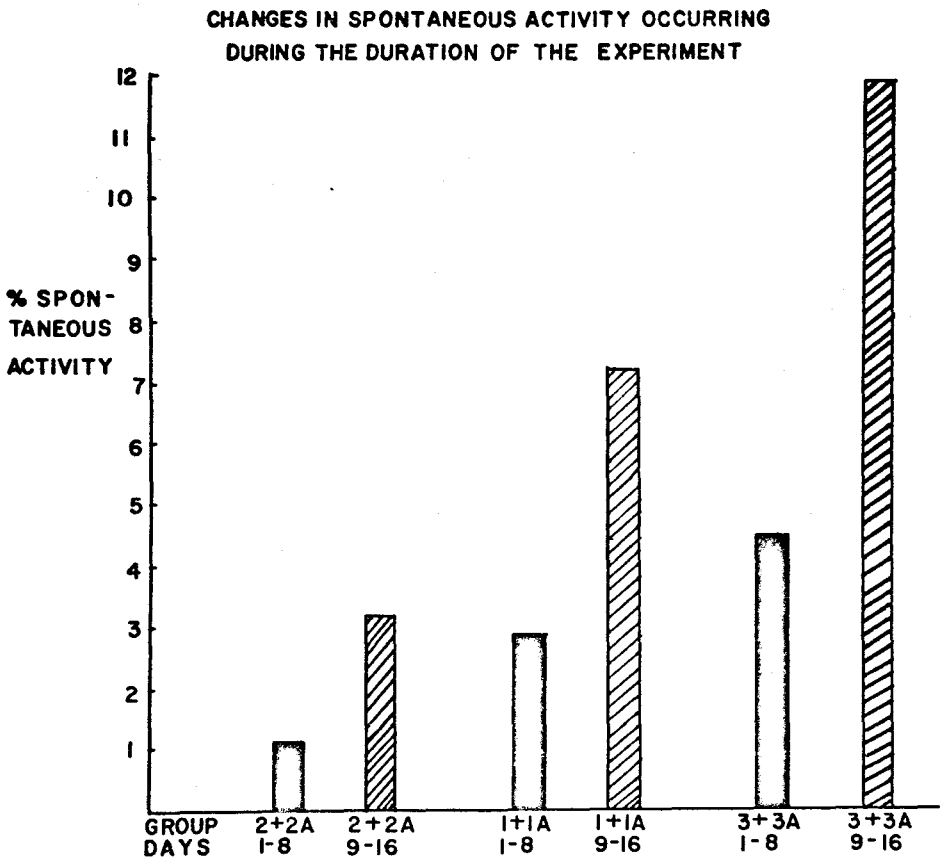

CHANGES IN SPONTANEOUS ACTIVITY OCCURRING
DURING THE DURATION OF THE EXPERIMENT
% SPON-
TANEOUS
ACTIVITY
12
11
10
9
8
7
6
5
4
3
2
1
GROUP
DAYS
2+2A
1-8
2+2A
9-16
1+1A
1-8
1+1A
9-16
3+3A
1-8
3+3A
9-16

FIGURE 41

LEGEND FOR FIGURE 41.

This figure depicts the difference in spontaneous activity observed between days one thru eight, solid bars, and days nine thru sixteen, striped bars, for each group in Experiment 12. Groups with the room lights on are pooled with the others since the changes observed are in the same direction. The abscissa indicates the corresponding groups and trial blocks. Group I and Group IA had the CS overhead in the safe chamber. Group II and Group IIA had no CS cue. Group III and Group IIIA had the CS in the safe chamber flashing at twice a second. The "A" suffix indicates that the room lights were on. One can see that in all cases the level of spontaneous activity increased in the later half of the experiment, days nine thru sixteen. Spontaneous activity is synonymous with spontaneous crossings. See Table 17 for statistics.

TABLE 17

SIGNIFICANCE OF CHANGES IN THE OCCURRENCE OF SPONTANEOUS CROSSINGS

GROUPS	OCCURRENCE TRIALS 1-5	OCCURRENCE TRIALS 6-10	t	p	SIG
2 & 2A	0.0129	0.0266	1.39	>0.1	N.S.
1 & 1A	0.0372	0.0444	0.490	>0.1	N.S.
3 & 3A	0.0483	0.0871	2.00	<0.05	Sig

GROUPS	OCCURRENCE DAYS 1-8	OCCURRENCE DAYS 9-16	t	p	SIG
2 & 2A	.0124	.0315	1.86	>0.05	N.S.
1 & 1A	.0290	.0720	2.74	<0.01	Sig
3 & 3A	.0450	.1190	4.24	<0.001	Sig

LEGEND FOR TABLE 17

The upper portion of the table supplies the statistical evaluation of the occurrence of spontaneous crossings in trials one thru five and trials six thru ten for results depicted in Figure 40. The lower portion of the table supplies the statistical evaluation for the same groups but comparing days one thru eight and days nine thru sixteen, as described in Figure 41. The Binomial t-test was used. Groups 2 and 2A had no CS. Groups 1 and 1A had the CS overhead in the safe chamber. Groups 3 and 3A had the flashing CS in the safe chamber at eye level. N.S. equals not significant and SIG equals statistically significant if p was <0.05. Only one group showed learning on a daily trial basis, that is Group 3 and 3A. However only the groups with the CS showed adaptive learning on a daily basis as depicted in the lower part of the table. Active adaptive learning was considered to occur when the frequency of occurrence of spontaneous crossings was significantly increased either on a trial or a daily basis.

DISCUSSION

Although there is much research, there is still little knowledge about the actual biological mechanisms of learning. It is even difficult to develop a rigorous definition of the process. Any personal difinition of learning is dependent upon one's training. For example, an experimental psychologist might try breaking down learning into its component parts. On the other hand, a general systems theory approach would be holistic, in that all of the various parts of an organism would be included, and learning would be considered to be a property of the entire biological or even abstract mechanical system. At present, perhaps the definition of learning ought to be general enough to encompass all forms of learning such as imprinting, one trial learning, insight learning and latent learning. The animal psychologist in his definition might try to be too specific, to exclude other processes that resemble learning such as habitation, fatigue, maturation, changes in receptors and effectors. The general system's scientist however, might include in his definition such processes as mechanisms of learning, and then would incorporate these processes into their appropriate hierarchial position with respect to all of the other mechanistic processes that ultimately are responsible for learning.

A simple definition of learning follows and can be considered to be a holistic definition. Learning may be defined observationally and objectively as; the progressive elaboration of a behavioral pattern through interaction with a particular

structuring of the environment; the behavioral pattern may or may not be novel, and the probability of occurrence of this behavioral pattern must increase or decrease upon repeated interaction with the specific environmental structure. This view of learning included imprinting, one-trial learning, insight learning and latent learning. This definition of learning also includes elaborations which would be due to native response tendencies, maturation, and temporary states such as fatigue, habituation, or alterations in receptors and effectors. However, all of these aforementioned elaborations of learning would be excluded from the psychologist's definition.

One cannot overlook the fact that the mechanisms of learning may differ within the same organism and amongst the different genera and species. In addition, any mechanism of learning must be a property of the total system and not restricted to any specific part.

As an example of the psychologists' attempts to subdivide and categorize learning, the following are considered by them to be special cases and should not be considered, they argue, to be part of the process of learning: native response tendencies, maturation, and temporary states such as fatigue, habituation, or alterations in receptors and effectors. To the psychologist the only phenomena which may be confused with learning are: sensitization (Hilgard and Marquis, 1940), backward conditioning, and pseudo conditioning. Sensitization is a response which increases

in frequency when the CS is paired with a UCS, but which does not resemble the unconditioned response (U.R.). Backward conditioning occurs when the behavior changes in response to a paradigm where the UCS is presented prior to the CS both having the same duration and intervening interval. Pseudo conditioning occurs when the CS-UCS pair are presented at either random duration or sometimes reversed, and a greater response is observed to the CS after the UCS has been given a number of times. Therefore, one can see that the experimental psychologists may have excluded from consideration some possible mechanisms for learning merely on the basis of the experimental techniques which they employ to study learning. One might argue that the experimental psychologists have allowed their techniques to give them a less exact concept of learning.

LEARNING IN FROGS AND TOADS: RELATIONSHIP BETWEEN TRIAL FORMAT, REST PERIODS AND ACTIVE AVOIDANCE LEARNING AS MEASURED BY CHANGES IN CHAMBER EXIT TIMES

The first experiments with BF_1, BF_2 and BF_3 had minimal controls. However, these experiments revealed an important aspect of the toad's response which supports Yerkes (1903b), who indicated in his reaction time studies, that the "freshness" of the animal was important for learning to be observed. Figures 6, 8 and 10 contain the supporting results. It can be seen from these same figures that all animals responded quite differently, yet these first experiments with BF_1, BF_2, and BF_3

supported Yerkes' original observation that no animal responded with pure avoidance. However, with the inclusion of rest periods, as in Figure 6, the animal showed lower exit times than the preceding trial on those trials which immediately followed the rest periods. It was hypothesized that the rest periods improved performance by allowing the animal to recover from any habituation, fatigue, or emotional factors which tended to make the toad inflate and passively avoid rather than actively avoid the shock.

Figure 8 is concerned with the conditioning of toad BF_2 which underwent conditioning without rest periods under the test/train/test paradigm. After the procedural change a drop in exit time values also occurred as it did for BF_1 on trials one, six, and sixteen; these exit time values are among the lowest values. However, there was one major difference between the two toads, BF_1 and BF_2. This difference was that trials other than trials one, six, and sixteen also had low exit time values. These low values occurred in trials four, twelve, and seventeen. The low values in these later trials seemingly were unrelated to the changes in the training procedure. To test whether the rest period or the training protocol was responsible for the observed decreases in exit time for trials six and sixteen, another animal, BF_3, was tested.

The protocol for BF_3 called for training trials only and excluded rest periods. Figure 10 illustrates the results for BF_3. It is quite obvious from the figure that no decreases in

exit time occurred in trials six and sixteen. The results of all three animals indicate that both the rest period and the shift in training procedure aided in maintaining the faster performance of BF_1 and BF_2 on trials six and sixteen.

It appeared from these data that the change in training procedure produced the greatest change in behavior; however, it was also necessary to evaluate the contribution of rest periods because it was felt that these also contributed to the observed behavioral changes. Therefore, the subsequent experiment with BA_2 and BF_5 evaluated the influence of rest periods within the confines of a constant training procedure.

The results of the experiment with BA_2 and BF_5 support the concept that a rested toad is more apt to learn an adaptive response. In both experiments the procedure consisted of training trials only and a rest period every ten trials. In these studies the lowest exit time value always occurred on the first trial of each block of ten trials; see Figure 16. These data indicate therefore, that the rest period played a great part in maintaining a consistent active avoidance response. If one were to apply these results in selecting the appropriate intertrial interval, it could be said that three minutes would be too short since it does not restore the response to the initial level. A complication enters into this analysis since a different training procedure was initiated after the rest periods with BF_1 and BF_2. However, in the experiments with BF_5 and BA_2 a rest period of

twenty minutes seems appropriate to restore responses to the initial level. In these cases, the training procedures remained constant. Therefore, one might conclude that the optimum inter-trial interval for avoidance conditioning ranges between three and twenty minutes, and could be biased towards the shorter time period if all trials were not the same.

One additional factor is evident in Figure 5 regarding the performance of BF_1. A strong negative correlation existed between the performance during the first test trials and the subsequent trials. It can be seen on the left side of the figure that the average exit time value for trials one thru five was negatively correlated with the average exit time values for trials sixteen thru twenty. The Pearson Correlation Coefficient was 0.95. Furthermore, on the right side of Figure 5 the average exit time value for trials one thru five was also negatively correlated with trials six thru fifteen. The Pearson Correlative Coefficient was 0.86. This phenomena was not observed in the performance of BF_2 where the respective correlation coefficients were 0.37 and 0.67. If the correlation, which was a measure of the consistency of performance, became less significant when the rest periods were absent, as in the case of BF_2, then one might consider that the rest periods had a considerable influence on the animal's ultimate behavior.

The next experiment was designed to investigate whether different conditioned stimuli would lead to different exit time

values. Initially no differences in exit time values existed between the groups; see Table 1. However, after training Group I seemed to have a shorter overall exit time, while the other two groups were indistinguishable from each other; see Table 2. A shorter exit time would also be an adaptive response.

Another indication of learning would be a lower average exit time value in trials six thru ten compared to trials one thru five. Examined on such a basis none of the groups exhibited lower exit time values during the last five trials. Thus one must conclude on the basis of exit time data above that no learning took place. Furthermore, the lower exit time values for Group I could have been due to the fact that they were the first group trained. During the training of Group I, the grid was not wetted by urine and could not have been short circuited to any extent. However, after the first group was trained the wires were wet and current could have leaked to the circuit ground causing a reduction in stimulus intensity for the latter two groups. During subsequent experimentation wooden dowels were employed to raise the grid from its support and thereby allowed the urine to drain off. In addition, the training of the groups was interspersed throughout the day and this produced a more balanced design.

Further experiments indicated that exit time data was not a useful indicator of adaptive learning in these animals since

their responses were so variable from trial to trial that significant differences could not be detected.

The variability in exit time data also was evident in the training of Bufo americanus and Bufo cognatus; see Tables 8 and 9 . Thus one can conclude that adaptive learning of toads in these procedures cannot be detected by examination of the exit time data. Spontaneous crossing data will be extensively discussed as an indication of active avoidance learning in another section of this thesis; see p. 194.

EXPERIENCE WITH RANA PIPIENS.

For the most part the difficulties encountered by McGill (1960) have been seen in this work with Rana; see p. 94 . The additional behavioral observations of Yerkes (1903a) and Burnett (1912) are entirely adequate to describe what has been observed here. Yerkes (1903a) noted the speed with which Rana moved in their escape attempts. This was also quite evident in this research since they sprang forward at least two to three times as quickly as Bufo. Yerkes (1903a), Burnett (1912), and McGill (1960) observed that many Rana showed attempts at climbing as a method of escape; climbing behavior was also observed by this author.

However, some methods for overcoming the difficulties of conditioning Rana were also tried by this author. One modification in the training procedure limited the total amount of shock received by Rana. Thus a limited shocking of fifteen seconds,

maximum duration, appeared to be well tolerated and still maintained adequate response levels; see Figure 10. One other investigator (Boice, 1970) has also used limited shocking to effectively avoid the problems seen by McGill. On the other hand, the five minute rest periods which were employed with Rana did not materially level out the observed variability in Rana's response; see Figure 11. It seemed that a very long intertrial interval would have been a more effective procedural change. It was also observed that an alternating high and low level of shock was of value in maintaining the aversiveness of shock.

Rana pipiens exhibited a lack of responsiveness to shock after a few trials. In every case a higher level of shock restored the frog's response, that is, to a lower exit time value. Theoretically, increased shock levels should not improve a fatigued animal's response (McGill, 1960). Since the waning responsiveness of Rana pipiens to shock was improved by higher levels of shock, then this waning responsiveness could not have been due to fatigue. Thus, some other phenomenon such as stimulus desensitization, habituation, true maladaptive conditioning or pseudo conditioning might have accounted for Rana's decreased responsiveness to shock.

The answer to the problem of why Rana fails to respond to shock after a few trials may reside in the relative amount of Rana's behavior which is mediated entirely by spinal reflexes as compared to Bufo.

Being so sensitive to the shock, Rana might have responded with a reflex response much more readily than Bufo.

In Rana, such a reflex oriented control mechanism seems to be an appropriate model which fits the observed results and hypothesis of Yerkes (1903a). Yerkes noted three different responses and formulated them into a model hypothesis. The very fast and immediate responses were thought to be due to spinal reflexes. An intermediate response time was said to be due to the influences of instincts and being more like a reflex involving higher brain centers. Yerkes called this intermediate response the "psychological reaction time". The slowest responses were said to have been due to voluntary or deliberate activity which involved the more complete use of higher level activity in a non-reflex sense. The slowest responses could also have been due to habituation. These slowest responses were often seen with stimuli of low intensity.

With regard to exit time data, Rana appeared to respond more rapidly when the room lights were on; see Figure 12. Furthermore, the mean exit times observed in this work compares well with that which was reported by Boice (1970), as five to ten seconds. The average exit time value for Rana ranged from seven to ten seconds after shock onset which was observed in those experiments where the duration of shock was limited.

With regard to spontaneous crossing, Rana rarely exhibited any. However, since inadequate testing of this response was

carried out, one could not categorically describe the degree of spontaneous crossings that could have been observed in Rana.

In conclusion, this investigation found the following facts about two-way shuttle avoidance conditioning of Rana. First of all, Rana seems to be very sensitive to shock since frogs respond initially to shock very quickly. However, the frogs' response to shock changes rather soon as evidenced by their steadily increasing exit times. Rana however, will show a more stable response to shock when the duration of shock is limited to fifteen seconds. A very long intertrial interval, something greater than two and a half minutes might also be useful (Figure 12). In addition, the alternate application of high and low voltage shock also stabilizes performance. Certain conditioning procedures seemed to be more effective in a well illuminated environment. Furthermore, although spontaneous crossing was rarely observed, the above findings if incorporated into training procedures might make spontaneous crossing more pronounced. Thus it is possible to work around some of the problems which had previously served as roadblocks in the conditioning of Rana.

SPONTANEOUS CROSSINGS AS A MEASURE OF ADAPTIVE LEARNING AND ACTIVE AVOIDANCE

CHANGES IN THE FREQUENCY OF OCCURRENCE OF SPONTANEOUS CROSSINGS WITH EXPERIENCE

The occurrence of spontaneous crossings was an unexpected

event in this research. This spontaneous crossing phenomena was an adaptive behavioral pattern within the constraints of the experiment since it protected the animals from receiving a noxious stimulus for a longer period of time; it also reduced the number of shocking trials within a day. Thus, an increase in the frequency of occurrence of spontaneous crossing with continued training was considered a measure of adaptive behavior. The active avoidance aspect of spontaneous crossing makes it significantly and qualitatively different from the type of passive maladaptive learning that McGill (1960) described in Rana. The spontaneous crossing data reflected the active avoidance learning of anura much more consistently than did the exit time data.

Figures 18 and 19 depict the first indications that spontaneous activity actually increased, in frequency of occurrence, with progressive training. Moreover, in Figure 19 one can see that after two days without training, the animal forgot and its performance deteriorated to a lower frequency of spontaneous crossings. Since a greater frequency of spontaneous crossings occurred when the CS appeared in the safe chamber, this was chosen as the standard procedure in subsequent experiments.

INFLUENCE OF VARIOUS TRAINING PROCEDURES AND DESIGN CHANGES ON THE FREQUENCY OF OCCURRENCE OF SPONTANEOUS CROSSINGS IN BUFO FOWLERI

The previous paragraph indicated the results of an experiment in which the spontaneous crossings of Bufo fowleri were

observed and quantified. The main procedural difference between that experiment and all those which had preceded it was the removal of four shocking grid wires from the center passageway between the chambers. The removal of the wires was significant since it allowed sufficient spontaneous crossings to be observed during the training, so that the observer began to record their occurrence. This major change in the environment was maintained throughout all subsequent experiments.

The subsequent experiment was designed to investigate whether different conditioned stimuli could lead to different exit time values and different frequencies of spontaneous crossings. Three groups of five animals were tested. Group I had the CS appear overhead in the safe chamber. Group II had no CS, Group III had the CS at eye level in the safe chamber flashing at a rate of twice a second. The following observations were made with regard to the exit time data. When the three groups of animals were naive, no significant differences were observed in their mean exit times; see Table 1. An observation such as this assures one that the groups constituted a homogeneous population. Moreover, during training, Group II and Group III were not significantly different as determined by their respective mean exit time values (Table 2). However, during training Group I had the shortest mean exit time, which is confirmed statistically in Table 2, and Group I showed an increased variability in response during the later trials

(Figure 23). These data for Group I indicated that they exited the chamber faster than the other groups during training; however, the group failed to establish a trend of faster exit times from trial to trial.

Yerkes (1903b) reported that the escape times for the later five trials usually were greater than those of the first five trials; such a situation was observed in Group III (Table 3). However, if adaptive learning occurred, the mean exit time values for the last five trials ought to be less than that of the first five trials. Table 3 indicates that no group showed faster exit times in the last five trials. Therefore, on the basis of exit time data no group exhibited adaptive learning, and all groups confirmed Yerkes' (1903b) observation in Rana.

The spontaneous crossing data for these groups was inconsistent with the exit time data. If adaptive learning occurred, one would expect the occurrence of spontaneous crossing to increase either from day to day or from trial to trial. Figure 25 indicates a significant difference between the overall levels of spontaneous crossing seen in the different groups. In examining the day to day performance of each group, one can see that only Groups II and III showed increases in spontaneous crossing over the course of the experiment (Figure 24). However, the members of Group I, who were the fastest escapers, did not show any increase in the number of spontaneous crossings in their day to day performance; see Figure 24.

Another measure of learning within a group, entails a greater frequency of occurrence of spontaneous crossings in the later trials of the procedure as opposed to that which occurred in the first few training trials. Using such an analysis and comparing the degree of spontaneous crossing in trials one thru five and six thru ten, one finds that the members of Group I did, in fact, show more spontaneous crossings in the last five trials. This indicates that during the daily training sessions more spontaneous crossings occurred during the later trials. Thus, it is possible that the spontaneous activity and adaptive learning was being induced by experience with the procedure. However, it was possible that Group I's daily forgetting was so much that no apparent gains were made when the frequency of spontaneous crossings was plotted on a daily basis as in Figure 24. Furthermore, Group II and Group III did not show adaptive changes between the trials even though they displayed an improvement in daily performance (Figure 26). Therefore, Groups II and III showed a different quality of learning than Group I.

Why was there a lack of correlation between the exit time data and the spontaneous crossing data in this experiment? The answer may lie in the design of the experiment. Group I was trained first, then Group II, and finally Group III. After Group I was trained, the possibility exists that the wires were sufficiently doused with urine to produce some short circuiting

of the wires which could account for the higher exit times in the two subsequent groups. To control for this, subsequent experiments had wooden dowels beneath the chambers, so that urine could drain off the shocking grid. Furthermore, since training was in a darkened room, Group II, although it had no CS, may have performed well because of the even lighting in the environment. Moreover, Group II could have used the photocell light source as an orienting device. Group III had the most radically changing CS and theoretically the one which demanded more attention, yet they did not exhibit learning when measured on a trial to trial basis. The most effective CS in this procedure on a trial to trial basis was the overhead CS in the safe chamber, but it was ineffective in yielding consistent daily learning.

Since it was found that a large amount of the escape time was spent in climbing the chamber walls, it was felt that elimination of this behavior would help in the acquisition of an adaptive, active avoidance response. Therefore, in this and subsequent experiments, the wires on the interior side walls and center walls were covered with construction paper. In addition wooden dowels were employed to raise the grid floor of the chamber from its support. This enabled urine to drain from the grid and prevented any shorting of the wires that might have occurred in previous procedures. Appendix B describes the changes under Experiment 9. Three groups of three BF were

trained. Group I had the CS overhead in the safe chamber. Group II had no CS, Group III had the CS flashing at two per second at eye level in the safe chamber.

As can be seen in Figure 27, the groups responded in an almost random fashion without any clear cut patterns. The overhead CS group, Group I mimicked the previous results of an analagous group in Experiment 8. They exhibited a general tendency towards increased exit times later in training and also tended towards increased variability. The modification in procedure did not seem to have any great influence on performance in terms of exit time data.

With regard to spontaneous crossings, once again the control group, the no CS group, had a greater frequency of occurrence of spontaneous crossings than both of the experimental groups (Table 7). Again, this could be attributed to the even lighting and timing of the procedure, since without a CS visual accomodation to changes in light intensity did not have to occur during the training procedure. Habituation could account for the decreased spontaneous crossing seen in Group III.

In an attempt to utilize some possible phototactic responses as an aid to conditioning, this species, the interior decor was altered in the two subsequent experiments, 11 and 12; see Appendix B and Methods Section. Furthermore, since something was known about the toads phototactic responses the following findings by various investigators were utilized in redesigning

the interior of the shuttle box.

Phototactic responses had previously been involved in conditioning studies and were partially successful (Munn, 1940). Moreover, the temperatures, 20.5°C to 23.9°C, at which training took place indicated that toads would display a positive phototaxis (Martoff, 1967). Similarly, the fact that toads cannot distinguish between a lighted area and a white solid indicated that white walls would be useful to attract a toad. It was also known that frogs lost a dark preference when forced to choose (Boycott, 1964). Since forcing and temperature were working to induce a positive phototaxis, the side and center walls were lined with white construction paper. Thus, when the CS lit up the safe chamber, it appeared brighter and would be the area of choice for a toad that was exhibiting a positive phototaxis.

To test for involvement of the positive phototaxis, the CS was presented in either the shocking or the safe chamber. It was expected that the observed results for these two groups would be opposite. Furthermore, the group which had the CS in the safe chamber should show some evidence of learning since the safe chamber would be more brightly illuminated during the shocking. The room lights were kept off to make the contrast as strong as possible.

The results of five days of training support the expected results. With regard to exit time analysis, Figures 32 and 33 depict the clear cut differences between the groups. Group V

which had the CS in the safe chamber responded fastest followed by Group VI which had no CS cue. Finally, Group IV, with the CS overhead in the shocking chamber, appeared to be more erratic. In Table 13 the differences between each group are shown to be statistically significant by the unpaired one-tailed student t statistic.

In comparing the various groups for changes in their early performance, trials one thru five, to their later performance, trials six thru ten, the usual changes were found; see Figure 34. In every group the later trials had a mean exit time which was greater than the former. Table 14 quantifies these differences and examines their statistical significance. The unpaired, one tailed, Student t-test was used. It was found that all groups have differences that just bordered on being statistically significant. The "p" value was less than 0.1. However, the results were considered to be biologically important since they followed the pattern previously established in other studies with Bufo fowleri.

In analysing the spontaneous crossing data, one can see in Figure 35 that the greatest amount of spontaneous crossing occurred in Group V, the group with the fastest exit time. The relative position of activity and mean exit times are also matched by the other two groups. Table 15 indicates that the observed differences were statistically significant with the two tailed Chi Squared analysis. These results are consistent

with current thinking about conditioning and how it is shown experimentally.

Further analysis of spontaneous crossing data involved the comparison of its frequency of occurrence during the early and late trials of a training session. Figure 36 illustrates these differences. It is in this area also where the expected differences between the phototatic responses would become evident. It can be seen that Group IV animals, with the CS in the shocking chamber, showed less spontaneous crossings in later trials compared to the early trials. However, both Group VI and Group V animals, the control group and the CS in the safe chamber group respectively, showed changes which suggest adaptive learning. Table 16 illustrates that the changes observed in Groups IV and V were statistically significant, while the differences observed in Group VI were not found to be statistically significant.

However, Group V, with the CS overhead in the safe chamber, again appeared to have the best training procedure producing the lowest exit times, the highest overall level of spontaneous crossings, and the greatest change in the occurrence of spontaneous crossings with experience.

Since the no CS group also showed a certain amount of spontaneous crossings, it was felt that some important controls were missing from the experimental design. Therefore, it was decided to place rigid controls on the entire experiment. The storage

of animals was controlled with respect to the lighting and temperature cycles and the amount and quality of light under which they were stored. Furthermore, the influence of room light being on during training had to be incorporated into the design. Moreover, it was considered imperative to control the time of training such that animals were trained at exactly the same time each day. In addition, individual members of a group were staggered in their training throughout the day to minimize the influence of diurnal rhythms during training. In addition, it was felt that training must be continued on a daily basis for as long as possible to accumulate a greater number of measurements, and thereby reduce the influence of the observed variation. These concepts were realized in the following experiment which concluded the experimental work of this thesis.

The results of this experiment could be interpreted in relation to the room illumination. Figure 37 illustrates the performance of the controls, Groups II and IIA. One can see that the trend towards increased exit times is quite apparent as well as high variability in response from trial to trial. It can also be seen that Group IIA had a mean response time that was approximately one half second faster than the other group. One interpretation would be that the increased illumination aided in the visualization of the environment for the faster group.

With regard to the overhead CS, Group I and Group IA, it

can be seen that the response for Group I was less variable than the control. Furthermore, Group IA, with the room lights on, showed a variable response similar to the control groups. One can interpret this observation also in relation to the room lights, because when they were on they lessened the contrast that occurred when the CS light went on. Therefore, the overhead CS would be less effective during the lights on situation.(Fig 38)

The flashing CS groups, Figure 39 showed a closeness of fit for the exit time data between both Groups III and IIIA. Furthermore, the characteristic variability of exit time data for both Groups III and IIIA was much less than that of the controls. The room lights being on did not affect the average exit times to any large extent. This was reasonable since when the CS was at eye level it was more readily apparent to the toad than the mere change in chamber illumination produced by it when it was positioned in the safe chamber for Group I.

The analysis of spontaneous activity for this experiment was very revealing since there was an increase in SA occurring in all groups studied. This increase was observable in all "A" groups as well. The spontaneous activity of any group was observed to increase in two ways. First on a trial to trial basis (Figure 40) and secondly on a daily basis (Figure 41).

Therefore these results indicated that the toad, Bufo fowleri, integrated the presence of a conditioned stimulus into an adaptive pattern of behavior. Since this pattern of behavior

emerged with a greater frequency as the animal acquired additional experience with his environment, one could conclude that adaptive active avoidance learning occurred.

SPECIES DIFFERENCES IN ACTIVE AVOIDANCE LEARNING

Since the degree of ambient illumination affected the responses of a frog, Figure 12, an experiment was designed to give an indication as to whether this also affected the spontaneous crossing observed with Bufo. The experiment compared the performance of three species of Bufo. Since little was known about learning in these species, it was initially assumed that they all had equal ability. Observed differences were to be examined more fully in later experiments. Bufo americanus #6 (BA_6), Rana pipiens Vermont variety #4 (RPV_4), and Bufo marinus #5, (BM_5) were all trained with the room lights on. On the other hand, Bufo fowleri #12 (BF_{12}) and Bufo americanus #7 (BA_7) were trained with the room lights off.

The daily frequency of spontaneous crossings are illustrated in Figure 20. BA_6 and BM_5 were the only two animals that exhibited a consistent increase in the daily frequency of occurrence of spontaneous crossings. Aside from the distinct possibility of individual differences, these data suggest that having the room lights on improved the learning of the animals, since both BA_6 and BM_5 were trained with the room lights on. However, both BF_{12} and BA_7 were trained with the room lights off and neither exhibited a consistent trend towards learning.

Rana pipiens did not exhibit any spontaneous crossings. However, the training procedure was not designed with the appropriate parameters, as had been previously described to be more advantageous for use in the conditioning of Rana; see previous section of discussion.

When one examines the performance of these animals by the changes in exit times, Figures 21 and 22, the differences between them is again obvious. Both BA_6 and BM_5, who showed an increased frequency of spontaneous activity at the end of training exhibited faster exit times than would have been expected if no adaptive learning took place. Furthermore, both BA_7 and BF_{12} who had inconsistent results in their spontaneous crossings (Figure 20) also exhibited longer exit times (Figures 21 and 22). Thus, BA_7 and BF_{12} received more shock than if they maintained their responses at the level they exhibited on the first day of training. Therefore, on the basis of the exit time data and the lack of evidence for adaptive avoidance behavior, one can conclude that the net result of the behavioral responses of BA_7 and BF_{12} to the experimental environment was maladaptive for both animals. In comparing the spontaneous crossing and exit time data, one could say that the room light aided two individual members of different species in developing an adaptive avoidance response to aversive shock.

When some Bufo americanus were available they were tested along with some other Bufo fowleri in a subsequent experiment.

See Methods, Appendix B and Experiment 9 for details. Bufo americanus showed a greater frequency of spontaneous crossings than Bufo fowleri (Figure 29). Although the trends in exit time data from trial to trial appeared similar, the overall mean exit times of Bufo americanus were longer than those of Bufo fowleri.

Another species which was more active than Bufo fowleri became available and was tested. The species was Bufo cognatus. Experiment 10 was carried out with this species. Details of the experimental procedure are in Appendix B; they were the same as for Experiment 9.

This active species seemed to respond more in the classical way to avoidance conditioning. Figure 30 shows that the exit times tended to decrease with progressive experience for all groups. Table 10 also indicates that the change is statistically significant for only Group I with the overhead CS. Table 9 also indicates that with respect to exit times the groups are significantly different from each other. Similarly, the degree of spontaneous crossings were significantly different between the groups; see Table 11. Figure 31 indicates that the animals showed more spontaneous crossing in the later trials. These changes however were not statistically significant. These results partially support the premise recently published by Boice (1970) that the more active species are more adaptable to an active avoidance task.

Both Bufo americanus and Bufo cognatus exhibited a greater

frequency of occurrence of spontaneous activity in training as compared to Bufo fowleri. It might well be argued that the presence of the CS actually inhibited the development of adaptive learning rather than aided it, especially by comparison to controls. These and all previous experiments in which the no CS group performed better than the experimental groups suggested at least two possible explanations. The first is that the toads were responding to stimuli other than the CS, such as noises of the apparatus or movements of the researchers, and they were responsible for the changes. Secondly, one might argue that the homing behavior of toads, which is temporarily synchronized by light in the environment, was involved in their spontaneous activity. Furthermore, the CS could have with repeated presentations interferred with the synchronization of this homing mechanism such that exit times became longer, the total amount of spontaneous activity dropped, and any net changes in spontaneous activity became smaller.

The first possible explanation could only be ruled out by a demonstration of responses to the CS being greater than that observed for the situation without a CS. However, one could argue that in the situation where the CS was absent some external event set the biological clock in the animal, and that with sufficient experience the animal could anticipate environmental changes relative to his preset internal clock.

Furthermore, the elegant work of Landreth and Ferguson (1968)

gives one a possible mechanism which makes the second argument become a distinct possibility. It has been shown that external sources of light could set the biological clock in toads and frogs, and that their homing behavior is related to this clock (Landreth and Ferguson, 1968) and (Taylor and Ferguson, 1970). Therefore, one might propose that the conditioned stimulus lights might repeatedly reset the clock in these animals, and thereby interfere with their ability to temporarily relate to changes in their environment. Furthermore successful conditioning with the CS would depend upon a well established rhythm. This was observed in the strictly controlled experiment where light, temperature, and time of training were all controlled.

ROLE OF THE FOREBRAIN AND DIENCEPHALON IN ANURAN AVOIDANCE RESPONSES: THE PHARMACOLOGICAL TARGET OF UNKNOWN PHYSIOLOGY

Investigation by others has shown that the anuran's hind brain and spinal cord control its behavior entirely by reflexes. These researchers also implied that the forebrain and diencephalon are necessary for less rigid behavior patterns.

Burnett (1912) indicated that the decerebrate frog is a good model of chain reflexes. He also indicated that they could learn to avoid shock. Nobel (1942) also alludes to the great amount of reflex integration that exists in frog spinal cord. Ewert (1970) stated that a pretectal and dorsal thalamic lesioned animal was an alert and responsive automaton which does

not display any passive avoidance behavior. One could suspect that the forebrain is important in inhibiting spinal reflex responses and in producing avoidance response via inhibited movement. Drugs which affect the coordinated activity of the forebrain, diencephalon, and the hindbrain-spinal cord system and their relation to the inflation response might alter anuran, adaptive, active avoidance learning (Cann and Scudder, 1970).

RESEARCH POSSIBILITIES AND FUTURE

Further research in this area should revolve around the study of lesioned animals and the effects of pharmacological agents on normal and lesioned animals. Enucleated animals should be studied to investigate whether the extraoptic light receptors which are involved in homing behavior (Taylor and Ferguson, 1970) are also involved in this adaptive behavioral response observed during these conditioning studies. Other conditioned stimuli should also be investigated to establish if they could also serve as appropriate sensory stimuli to effect adaptive behavior. With the smaller neural mass, it should statistically be easier to examine the inter relationships of the learning mechanism in anura; however, the technical difficulties are still great. A fruitful mechanistic approach to the investigation of the physiological learning mechanism and the pharmacological modification of that mechanism would entail auto and cross correlation analysis of unit potentials from various sites with a specific, conditioned stimulus, evoked potential that was

directly associated with the adaptive response. Such an approach might give insight into the series of synapses involved in eliciting the response.

QUALITATIVE CRITERIA INDICATIVE OF CONDITIONING

Bullock and Quarton (1966) have presented a working definition of classical conditioning. "A stimulus class (CS) that before the conditioning procedure does not produce a response, or produces it with a low incidence will, after the conditioning procedure, produce that response with a frequency above some specific criterion. The conditioning procedure consists in pairing of the CS with another stimulus (US) that ordinarily produces an unconditioned response (UR). The paired presentation must be in a certain order (CS-US) with a CS-US interval within specific limits both with respect to mean duration and variability. The CR must meet criteria of resemblance to the UR". Similarly this definition may be adjusted by setting limits on the acquisition time, level of accomplishment etc.

These authors also have established some criteria by which one can compare an apparent instance of learning and determine if it can be considered as such. A discussion of these criteria and the applicability of the final experimental procedure follows.

Initially one must define the CS, US, CR and UR. Then the CS-US interval must be fixed. The CS is either the overhead light in the safe chamber or the flashing light in the safe

chamber. The room lights may be on or off. The CR should be a hop, jump, leap, or crawl-walk into the safe chamber. The UR is the same as the CR. As for the direction and variability of the CS-US interval, CS appears for three seconds ± 0.1 second prior to US. The US is accompanied by CS until UR occurs. The resemblance of UR and CR need not be absolute in this case, but must produce the same net displacement of the animal's body. Furthermore, the required change in frequency of the CR would be such that CR increases to a statistically significant level when the last half trials are compared to the first half trials on a daily or total trial basis.

"The CR must resemble the UR sufficiently so that sensitization could be ruled out" (Bullock and Quartan, 1966). This in essence has been accomplished by the very nature of the response. It is not considered to be pragmatically useful to restrict the CR to be an exact replicate of the UR since the UR varied in intensity and form; however, the result of its activity is defined. Sensitization is considered to be learning in the holistic sense, and does not need to be ruled out as a false positive.

One furthermore must demonstrate that learning does not occur in the absence of any one of the controlling parameters. The control group indicates that although some increase in the frequency of CR occurs it is either insignificant or is greatly superceded by the presence of CS. The fact that different CS stimuli can alter the final level of performance indicates that

the CS is the controlling factor. Some additional experimentation on the order suggested by Bullock and Quarton (1966) might also be useful to further solidify this point. That is, one may use a reversed order of CS-US presentation or set it up as a random occurrence instead of a defined pairing. Another method would be to incorporate a second equally conditionable CS into the procedure, but not to pair it with the US in any way, and then to demonstrate that the conditioned response does not change in relation to the placebo CS.

One must also be sure to demonstrate that no factors other than the ones specified could account for the behavioral change. For instance surgery, food deprivation, some environmental change or organismal change of which we may not be measuring may occur in synchrony with the procedure and thereby mimic learning. Some examples of organismic factors are aging, maturation, acclimation, change in satiation, state of deprivation, arousal, motivation, stress, attention, fatigue, cicadian rhythm, modification of receptors or effectors, pheronomes and gonadal state (Bullock and Quartan, 1966).

Furthermore, one must demonstrate that the apparent effective stimulus is in fact, the effective stimulus. This has been accomplished by utilizing the no CS group as a control for factors other than the defined CS and the UCS. The no CS group ruled out factors such as movements of the investigator and noises of the apparatus, both of which occurred in relation to

the experiment. I believe that the demonstration of an increase in spontaneous crossings over the duration of the experiment was a significant adaptive response, and if a non specific arousal response was responsible for the adaptive behavior then it was exactly that non specific response which had become conditioned.

A definite potential for all of these influences rests easily within the framework with which the conditioning of Bufo fowleri has been presented. Certainly, phenomena such as non specific responses, sensitization, environmental changes, and hormonal state still need to be explored as being possible contributing factors for the observed change in behavior. However, I have indicated that learning occurred since it was my impression that the observed behavior at times was too complex for a system which lacked plasticity. Objectively, however, one must say that a patterned behavior has been elaborated on the continued exposure to a strictly defined environment. Furthermore, although the toad has been viewed as an automaton simply controlled by spinal reflexes, this work indicates that toads also exhibit a plasticity in their behavior and reinforces the concept of plasticity in a mechanistic system.

CONCLUDING REMARKS: COMPARATIVE LEARNING THEORY AND LEARNING IN THE AMPHIBIA

In concluding this discussion I believe that two pertinent aspects still remain which must at least be mentioned. The first of these is of critical importance since it involves the

conceptulization of a comparative theory of psychology, behavior, and learning. Hodos and Campbell (1969) have pointed out the fallacy intrinsic to Bitterman's (1965) argument for the phylogenetic theory of learning ability becoming greater as one moves from fish to turtle to pigeon to rat to monkey and finally to man. Hodos and Campbell (1969) eloquently bring home the role of environmental niches in evolution and the subsequent development of parallel lines of evolution. Their suggestion, which should be well taken, prescribes that if one is to pursue a theory of comparative psychology one is better off in understanding the role of environmental niches in evolution, and one should study closely related species first, rather than trying to compare distant species. They also suggest that one should forget about the concepts of higher and lower animals. Some early examples of the misapplicability of comparative psychological studies are given by Yerkes' (1903a) reference to Triplett (1901) relating frog and perch learning. Yerkes (1903a) also recalls his own work (Yerkes, 1901a) in comparing the turtle to the frog. Nobel (1942) and Franz (1927) also compare the learning abilities in relative distant species. Thus I suggest that further studies in the Anura be undertaken with related species before generalizations about learning mechanisms are made to unrelated species.

The second aspect of importance concerns itself with the general question of the presence of learning as a trait in

amphibians. I believe that the view of the frogs and toads as simple automatons without plasticity (see introduction to this thesis) is well justified by initial observation and experiment, although sufficient evidence has been previously accumulated to show that these animals readily formed feeding associations. Unfortunately, the level of control in these experiments as well as the number of animals involved still needs to be strengthened to solidify their observations. Moreover, there exists now sufficient evidence relative to avoidance conditioning by Ray (1967 and 1970), Boice (1970), and Cann and Scudder (1970) to defend the fact that active avoidance also occurs in the Amphibia. Ray (1970) projects optimism by stating that the "low assessment of the learning capacities of amphibians is not justified". I concur from this research experience with Ray's statement that the poor performance observed in amphibian learning studies came from an inability to motivate these animals without inhibiting them.

The demonstration of an increased frequency of spontaneous crossings in the toads as systems which have previously been thought to be entirely devoid of any significant degree of behavioral plasticity is exciting and hopefully will stimulate much more research in this area.

SUMMARY

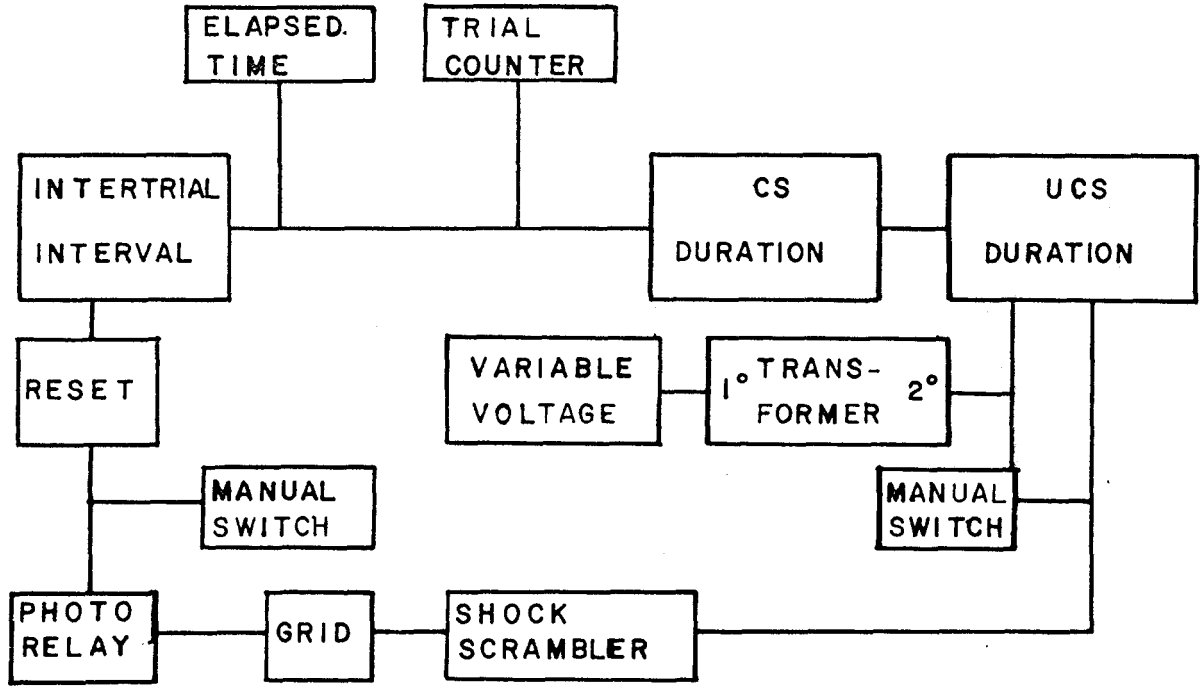
In summary the spontaneous crossing data meets the criteria established by Bullock and Quartan (1966) for demonstrating classical conditioning. The exit time data, because of great variability, are not sensitive indicators of learning in the two-way shuttle avoidance conditioning of Anura. The spontaneous crossing behavior is a response that has not been previously reported in toads under these or similar training conditions.

Other general findings with respect to spontaneous crossings:

1. Spontaneous activity increases with progressive experience.
2. Spontaneous activity was increased in these experiments by having the room well illuminated.
3. Certain stimuli which theoretically should elicit greater spontaneous activity do in fact elicit greater amounts of spontaneous activity.
4. Spontaneous activity occurs in response to shock alone, but any increase with additional training is not statistically significant.
5. This behavior is adaptive and does fit the definition of adaptive learning.

APPENDIX A

BLOCK DIAGRAM OF ELECTRICAL & MECHANICAL CONTROLS



APPENDIX B

ABBREVIATIONS USED IN APPENDIX B.

Tab	Number of table in which experimental results can be found
Fig	Number of figure in which experimental results can be found
Ex	Experimental number
Spec + Code	Species code and number code for animals used
Trials or Group	Trials with a particular paradigm or group designation (I, II, III, IV, V, VI)
ITI	Intertrial interval duration in seconds
UCS	Unconditioned stimulus parameters
V	Voltage
D	Maximum duration of presentation in seconds till exit or termination of trial
CS	Conditioned stimulus parameters
C	Color (R = red, W = white, D = dark, BU = blue)
D	Duration of presentation in seconds
CSP	Manner of presentation of the CS
O	Overhead
N	No CS was presented
F	Flashing CS at eye level
SH	Presented in the shocking chamber
SF	Presented in the safe chamber
L	Condition of the room lights (+) on and (-) off
S	Color of construction paper covering the side walls (E = no covering, W = white, BU = blue, BK = black)

ABBREVIATIONS USED IN APPENDIX B.

C	Color of construction paper covering the center wall (E = no covering, W = white, BU = blue, BK = black)
F	Color of construction paper covering the far wall (E = no covering, W = white, BU = blue, BK = black)
B	Color of construction paper laying under the base (E = no covering, W = white, BU = blue, BK = black)
T/D	Number of trials per day
D/T	Number of days of training
Av	Time in seconds. Any exit time less than or equal to the listed value constituted an avoidance response while any value greater than the listed value constituted an escape response.

e F	T/D	D/T	Av	Comments
E	10	8	11	Test/Train/Test paradigm with 3 min. rest periods between testing and training trials. CS & UCS were
	10		0	presented simultaneously in training trials.
"	10	9	11	As above only without rest periods.
	10		0	
"	20	8	0	Training trials only, no rest periods, no barrier.
"	10	4	11	Same as BF1 above but with a central barrier, white.
	10			
"	10	4	11	Same as BF2 above but with a central barrier.
	10			
"	20	1	2	No difference between chambers.* left = R,right = Bu.
"	20	2	2	20 min. rest periods between ea. block of ten trials.
"	40	1	2	20 min. rest periods between ea. block of ten trials.
"	20	3	3	First experiment with Rana.
BK	60	2	2	Room lights were on for the last trials on day 2.
"	90	1	3	More shock improved performance.
"				
"	60	1	3	Five min. rest periods were given every five trials.
"	90	3	3	Massed trials, did not improve performance.
Bu	50	5	3	Removed the 4 wire hurdle, started at the same time
"	"	"	"	each day.
"	10-20	5	3	Effect of room lights no wire hurdle in all subse-
"	"	"	"	quent
"	"	"	"	
"	"	"	"	
"	"	"	"	

Tab	Fig	Ex	Spec and Code	Trial or Group	ITI	UCS V	UCS D	CS C	CS D	CSP O	CSP N	CSP F	Grid L	Coverings Inside S	Coverings Inside C	Coverings Inside F	Coverings Outside S	Coverings Outside B
	5,6	1	BF1	TEST	60	7	60	R	6	Sh	-	-	+	E	E	E	E	E
				TEST				D	5		X							
				TRAIN	"	"	"	R	60	"	-	-	"	"	"	"	"	"
	8	"	BF2	TEST	"	"	"	R	6	"	-	-	"	"	"	"	"	"
				"				D	5		X							
				TRAIN	"	"	"	R	60	"	-	-	"	"	"	"	"	"
	10	"	BF3	TRAIN	"	"	"	"	60	"	-	-	"	"	"	"	"	"
	7	2	BF1	TEST	"	"	"	R	6	"	-	-	"	"	W	"	"	"
				TEST				D	5		X							
				TRAIN	"	"	"	R	60	"	-	-	"	"	"	"	"	"
	9	2	BF2	TEST	"	"	"	R	6	"	-	-	"	"	"	"	"	"
				TEST				D	5		X							
				TRAIN	"	"	"	R	60	"	-	-	"	"	"	"	"	"
		3	BA1		30	"	"	*	2	"	-	-	"	"	E	"	"	"
	16	"	BA2	- - -	*	4	60	W	"	"	-	-	"	"	"	"	"	"
	15	"	BF5	- - -	60	"	"	"	"	"	-	-	"	"	"	"	"	"
	11	4	RP1	- - -	60	3	300	"	3	"	-	-	"	"	"	"	"	"
	12	5	RP3	- - -	150	4	"	"	2	"	-	-	-	"	W	"	BK	BK
	13	"	RPV2	1-67	60	"	6	"	3	"	-	-	"	"	Y	"	"	"
				68-90	"	4&8	15	"	"	"	-	-	"	"	"	"	"	"
	14	"	RPV3	- - -	"	4	6	"	"	"	-	-	"	"	"	"	"	"
	17	7	BF6	- - -	"	7	60	"	2	"	-	-	"	"	E	"	"	"
	18	"	BF6	- - -	"	"	"	"	3	"	-	-	"	"	"	"	"	"
	19	"	BF11	- - -	"	"	"	"	"	Sa	-	-	"	"	"	"	"	"
	20	6	BA6	- - -	"	"	"	"	"	Sh	-	-	+	"	"	"	"	"
	21	"	BA7	- - -	"	"	"	"	"	"	-	-	-	"	"	"	"	"
	22	"	RPV4	- - -	"	"	"	"	"	"	-	-	+	"	"	"	"	"
		"	BF12	- - -	"	"	"	"	"	"	-	-	-	"	"	"	"	"
		"	BM5	- - -	"	"	"	"	"	"	-	-	+	"	"	"	"	"

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e F	T/D	D/T	Av	Comments
Bu " "	10-20	5 " "	3 " "	Investigate procedural differences.
Bu " " " " "	10-20	5 " " " " "	3 " " " " "	Wooden dowels were used in this expt. and all following. Inside wires were covered to prevent climbing. Both species and procedural differences were examined.
Bu " "	10-20	5 " "	3 " "	Investigated the spontaneous activity of another species.
BK " "	10-20	5 " "	3 " "	Black and white contrast was incorporated in this and subsequent experiments.
BK " " " " "	10-20	16 " " " " "	3 " " " " "	Investigated the effect of room light and additional training. Environmental controls were established for storage between trials.

Tab	Fig	Ex	Spec and Code	Trial or Group	ITI	UCS V	UCS D	CS C	CS D	CSP O	CSP N	CSP F	CSP L	Grid Coverings Inside S	Inside C	Inside F	Outside S	Outside B
1,2,	23,24	8	BF(5)	I	60	7	60	W	3	Sa	-	-	-	E	E	E	BK	BK
3,4	25,26	"	"	II	"	"	"	-	-	-	X	-	"	"	"	"	"	"
		"	"	III	"	"	"	W	3	-	-	Sa	"	"	"	"	"	"
5,6	27,28	9	BF(3)	I	"	"	"	"	"	Sa	-	-	"	BK	BK	BU	BK	BK
7,8	29	"	BA(2)	I	"	"	"	"	"	"	"	"	"	"	"	"	"	"
		"	BF(3)	II	"	"	"	-	-	-	X	-	"	"	"	"	"	"
		"	BA(2)	II	"	"	"	"	"	"	"	"	"	"	"	"	"	"
		"	BF(3)	III	"	"	"	W	"	-	-	Sa	"	"	"	"	"	"
		"	BA(2)	III	"	"	"	"	"	"	"	"	"	"	"	"	"	"
9,10,	30	10	BC(3)	I	"	"	"	"	"	Sa	-	-	"	"	"	"	"	"
11,12	31	"	BC(3)	II	"	"	"	"	"	-	X	-	"	"	"	"	"	"
		"	BC(3)	III	"	"	"	"	"	-	-	Sa	"	"	"	"	"	"
13,14	32	11	BF(5)	IV	"	"	"	"	"	Sh	-	-	"	W	W	BK	"	W
15	33,34	"	BF(5)	VI	"	"	"	-	-	-	X	-	"	"	"	"	"	"
	35,36	"	BF(5)	V	"	"	"	W	3	Sa	-	-	"	"	"	"	"	"
	37,38	12	BF(3)	I	"	"	"	"	"	Sa	-	-	-	"	"	"	"	"
	39,40		BF(2)	IA	"	"	"	"	"	"	-	-	+	"	"	"	"	"
	41		BF(3)	II	"	"	"	-	-	-	X	-	-	"	"	"	"	"
			BF(2)	IIA	"	"	"	"	"	"	"	"	+	"	"	"	"	"
			BF(3)	III	"	"	"	"	"	-	-	Sa	-	"	"	"	"	"
			BF(2)	IIIA	"	"	"	"	"	-	-	Sa	+	"	"	"	"	"

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APPROVAL SHEET

The thesis submitted by Frank J. Cann has been read and approved by a committee composed of Alexander G. Karczmar, M.D., Ph.D., Robert S. Schmidt, Ph.D. and Charles L. Scudder, Ph.D. (Chairman).

The final copies of the thesis have been examined by the chairman of the examining committee and his signature which appears below verifies that all the necessary changes have been incorporated and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

Oct. 25, 1973
Date

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Signature of Committee Chairman